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CURRENT DEVELOPMENTS AND THEMATIC REVIEWS IN HEALTH SCIENCES

Editors
Assoc. Prof. Emre Gökçen, MD.
Assoc. Prof. Ekim Sağlam Gürmen, MD.

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Assoc. Prof. Emre Gökçen, MD.

Assoc. Prof. Ekim Sağlam Gürmen, MD.

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CHAPTER 1

Digital Dentistry and Virtual Surgical Planning in Maxillofacial Reconstruction

Özge Özdal Zincir[1]

[1] Assoc. Prof. Dr., Istanbul University- Cerrahpasa, Faculty of Dentistry, Department of Oral and Maxillofacial Surgery ORCID: 0009-0000-9423-5764

Introduction

The integration of digital technologies into oral and maxillofacial surgery has fundamentally transformed diagnosis, treatment planning, surgical execution, and postoperative rehabilitation. Over the last two decades, advances in digital dentistry, virtual surgical planning (VSP), computer-aided design/computer-aided manufacturing (CAD/CAM), intraoral scanning, three-dimensional (3D) imaging, additive manufacturing, and navigation systems have enabled highly precise and patient-specific treatment approaches in maxillofacial reconstruction [1].

Maxillofacial defects resulting from trauma, oncologic surgery, congenital deformities, osteonecrosis, and severe atrophy often present complex anatomical and functional challenges [2]. Conventional reconstructive techniques historically relied on two-dimensional imaging, manual model surgery, intraoperative freehand contouring, and surgeon experience. Although these methods remain clinically valuable, they may be associated with prolonged operative time, reduced precision, unpredictable esthetic outcomes, and increased morbidity.

Digital dentistry has introduced a workflow-centered reconstructive philosophy in which imaging, planning, simulation, manufacturing, and prosthetic rehabilitation are integrated into a comprehensive virtual environment [3]. Such workflows allow surgeons to evaluate anatomy three-dimensionally, simulate osteotomies, fabricate patient-specific devices, and optimize both functional and esthetic reconstruction before entering the operating room.

Virtual surgical planning has become one of the most important innovations in modern maxillofacial reconstruction. VSP combines high-resolution imaging, digital segmentation, CAD software, and 3D visualization tools to create precise surgical plans tailored to the patient's anatomy [4]. These technologies improve communication between surgeons, prosthodontists, radiologists, biomedical engineers, and laboratory technicians while enhancing predictability and efficiency.

The combination of digital planning and CAD/CAM fabrication has additionally enabled the development of patient-specific implants, cutting guides, reconstruction plates, surgical splints, and prosthetic frameworks [5]. Such personalized solutions significantly improve reconstruction accuracy and reduce intraoperative adaptation requirements.

Three-dimensional printing technologies have further expanded the applications of digital dentistry in reconstructive surgery. Anatomical models generated through additive manufacturing allow surgical simulation, preoperative rehearsal, patient education, and fabrication of customized surgical devices [6].

Another major advantage of digital workflows involves integration of prosthetically driven planning principles. Modern reconstruction increasingly emphasizes restoration not only of facial contour but also of occlusion, mastication, speech, and implant-supported dental rehabilitation [7].

Despite these advances, important limitations remain regarding cost, software complexity, learning curve, manufacturing time, and accessibility. Nevertheless, ongoing improvements in imaging technologies, software automation, and digital integration continue to expand the role of virtual workflows in oral and maxillofacial reconstruction.

This chapter reviews the principles, technologies, clinical applications, advantages, limitations, and future directions of digital dentistry and virtual surgical planning in maxillofacial reconstruction.

Principles of Digital Workflow in Maxillofacial Reconstruction

Digital workflow in maxillofacial reconstruction refers to the integration of imaging acquisition, virtual planning, computer-assisted design, manufacturing technologies, and surgical execution into a coordinated digital treatment process [8].

The workflow generally begins with acquisition of patient-specific anatomical data using computed tomography (CT), cone-beam computed tomography (CBCT), magnetic resonance imaging (MRI), facial scanning, or intraoral optical scanning [9]. These imaging modalities provide highly detailed three-dimensional anatomical information essential for virtual reconstruction.

CT imaging remains the gold standard for evaluation of craniofacial hard tissues because of its excellent spatial resolution and ability to generate Digital Imaging and Communications in Medicine (DICOM) datasets [10]. CBCT offers lower radiation exposure and is particularly useful in dentoalveolar applications.

Digital segmentation is subsequently performed using specialized software to isolate anatomical structures such as bone, teeth, tumors, vascular structures, or soft tissues [11]. Accurate segmentation is critically important because all subsequent virtual planning depends on the precision of the digital model.

After segmentation, CAD software allows surgeons and engineers to manipulate anatomical structures virtually. Osteotomies, resections, reconstructions, implant placement, and prosthetic positioning may be simulated in a three-dimensional environment [12].

One of the major principles of digital reconstruction involves backward planning. Instead of reconstructing anatomy solely according to skeletal appearance, modern workflows frequently begin with the desired prosthetic and functional outcome [13]. Bone reconstruction is subsequently planned to support optimal implant placement and prosthetic rehabilitation.

Digital workflows additionally facilitate interdisciplinary collaboration. Oral surgeons, prosthodontists, oncologic surgeons, radiologists, and biomedical engineers may simultaneously evaluate and modify treatment plans within virtual platforms [14].

Data obtained from virtual planning may subsequently be transferred to CAD/CAM manufacturing systems for fabrication of patient-specific implants, cutting guides, occlusal splints, and reconstruction plates [15].

Accuracy and reproducibility represent essential components of successful digital workflow implementation. Errors in imaging acquisition, segmentation, software processing, or manufacturing may compromise surgical outcomes [16]. Consequently, quality control remains critically important throughout all stages of digital planning and execution.

Imaging Technologies in Digital Reconstruction

Advanced imaging technologies form the foundation of digital maxillofacial reconstruction. Precise anatomical visualization is essential for accurate diagnosis, virtual planning, and fabrication of customized surgical devices [17].

Computed tomography remains one of the most important imaging modalities in reconstructive surgery because of its high-resolution evaluation of craniofacial skeletal anatomy [18]. Multislice CT allows detailed visualization of bone morphology, tumor extent, fracture patterns, and donor sites.

Cone-beam computed tomography has become increasingly important in dentoalveolar and implant-related applications because of lower radiation dose and excellent hard tissue visualization [19]. CBCT datasets are frequently integrated into implant planning and prosthetic workflows.

Magnetic resonance imaging is particularly valuable for evaluation of soft tissue pathology, vascular structures, temporomandibular joint disorders, and oncological extension into soft tissues [20]. Fusion of MRI and CT datasets may significantly improve surgical planning in complex cases.

Intraoral optical scanning has revolutionized digital dentistry by enabling highly accurate acquisition of dental arches and occlusal relationships without conventional impression materials [21]. Digital impressions may be directly integrated into virtual reconstruction workflows.

Facial scanning technologies additionally allow three-dimensional evaluation of facial soft tissues and esthetic relationships [22]. Combining facial scans with skeletal imaging facilitates more comprehensive reconstruction planning.

Image fusion techniques have become increasingly sophisticated. DICOM datasets from CT or CBCT may be merged with stereolithography (STL) files

obtained from intraoral scanners and facial scanners [23]. Such multimodal integration allows highly accurate virtual treatment simulation.

Navigation imaging and dynamic guidance systems additionally provide real-time intraoperative localization and accuracy verification during reconstructive procedures [24].

Despite technological progress, imaging limitations remain important considerations. Motion artifacts, metal artifacts, segmentation inaccuracies, and image distortion may compromise digital accuracy [25]. Careful imaging protocol selection and quality assurance are therefore essential.

Virtual Surgical Planning

Virtual surgical planning represents one of the most transformative developments in contemporary maxillofacial reconstruction. VSP allows surgeons to perform digital simulation of complex procedures before surgery, thereby improving precision, predictability, and interdisciplinary communication [26].

The VSP process generally begins with creation of a three-dimensional anatomical model based on patient imaging data. Surgeons may subsequently simulate tumor resection, fracture reduction, osteotomies, graft positioning, implant placement, and occlusal correction within the virtual environment [27].

One of the most important advantages of VSP involves preoperative evaluation of anatomical limitations and reconstruction feasibility. Potential complications and technical difficulties may often be identified before surgery [28].

Mirroring techniques are frequently used in unilateral deformities or trauma cases. The unaffected side may serve as a digital template for reconstruction of the affected region, improving facial symmetry and esthetic outcomes [29].

In oncologic surgery, VSP allows precise planning of resection margins and reconstruction strategies. Fibula free flap reconstruction has particularly benefited from virtual planning because osteotomies and graft positioning may be simulated accurately before surgery [30].

Occlusal relationships and prosthetic rehabilitation may additionally be integrated into the planning process. Implant positioning and prosthetic design may therefore guide skeletal reconstruction rather than being considered only after surgery [31].

Virtual planning significantly improves interdisciplinary collaboration. Surgeons and prosthodontists may communicate treatment objectives more effectively within digital platforms [32]. Such collaboration is particularly important in implant-supported rehabilitation cases.

Several studies have demonstrated that VSP may reduce operative time, ischemia duration in free flap surgery, intraoperative uncertainty, and need for plate adaptation [33]. Improved efficiency may additionally reduce anesthesia duration and hospitalization costs.

Despite substantial advantages, VSP remains associated with certain limitations. Software costs, learning curve, dependence on imaging quality, and manufacturing delays may limit accessibility in some settings [34]. Furthermore, intraoperative anatomical variation may occasionally differ from the preoperative virtual model.

Nevertheless, VSP has become an increasingly central component of precision-based maxillofacial reconstruction.

CAD/CAM Technologies in Maxillofacial Reconstruction

Computer-aided design and computer-aided manufacturing technologies have become indispensable components of modern digital maxillofacial reconstruction. CAD/CAM systems allow the transformation of virtual surgical plans into precise physical surgical tools and patient-specific reconstructive devices [35].

Computer-aided design software enables surgeons and biomedical engineers to create customized virtual models based on patient anatomy. Osteotomy guides, reconstruction plates, occlusal splints, prosthetic frameworks, and patient-specific implants may all be digitally designed according to individualized treatment objectives [36].

Computer-aided manufacturing subsequently converts these digital designs into physical structures using subtractive or additive manufacturing methods. Milling technologies and three-dimensional printing systems are the most commonly used fabrication techniques in maxillofacial surgery [37].

Patient-specific cutting guides represent one of the most widely utilized CAD/CAM applications. These guides transfer virtual osteotomy plans accurately into the surgical field and improve precision during tumor resection, orthognathic surgery, and free flap reconstruction [38].

Customized reconstruction plates are another important innovation. Conventional reconstruction plates frequently require extensive intraoperative contouring, increasing surgical time and reducing accuracy [39]. CAD/CAM-fabricated plates may be pre-adapted precisely to the patient's anatomy and virtual reconstruction plan.

Mandibular reconstruction using fibula free flaps has particularly benefited from CAD/CAM workflows. Fibular osteotomies, mandibular contouring, and plate adaptation may all be digitally planned before surgery [40]. Such

approaches improve facial symmetry, occlusal rehabilitation, and reconstructive precision.

Patient-specific implants fabricated through CAD/CAM technologies are increasingly used in orbital reconstruction, cranial defects, mandibular defects, temporomandibular joint reconstruction, and severe facial asymmetry [41]. Titanium remains the most commonly used material because of its excellent biocompatibility and mechanical properties.

Another major advantage of CAD/CAM systems involves improved prosthetically driven rehabilitation. Implant-supported prosthetic reconstruction may be integrated into the reconstructive workflow from the earliest planning stages [42].

Although CAD/CAM technologies improve surgical precision and efficiency, limitations include high manufacturing cost, software dependency, and potential inaccuracies associated with digital conversion processes [43]. Nevertheless, technological refinement continues to improve accessibility and clinical reliability.

Three-Dimensional Printing in Digital Reconstruction

Three-dimensional printing technologies have significantly expanded the capabilities of digital reconstruction by enabling fabrication of highly accurate anatomical models and customized surgical devices [44].

Additive manufacturing creates physical objects through layer-by-layer deposition according to digital STL files generated during virtual planning [45]. Various printing technologies including stereolithography, fused deposition modeling, selective laser melting, and electron beam melting are currently used in maxillofacial applications.

Anatomical models are among the most valuable applications of 3D printing in reconstruction. Patient-specific models allow surgeons to visualize complex anatomy, rehearse procedures, pre-bend plates, and improve interdisciplinary communication [46].

Surgical simulation using printed models may reduce intraoperative uncertainty and improve operative efficiency. Complex tumor resections, craniofacial deformity corrections, and trauma reconstructions may all be rehearsed preoperatively [47].

Three-dimensional printed guides additionally improve accuracy of osteotomies and implant placement. Cutting guides generated from virtual surgical plans may transfer digital precision directly into the operating room [48].

Patient-specific implants fabricated through metal additive manufacturing have become increasingly important in mandibular and craniofacial

reconstruction. Customized implants may improve anatomical adaptation and reduce intraoperative modification requirements [49].

Educational applications are also highly significant. Residents and trainees may benefit from realistic anatomical models that simulate patient-specific pathology and surgical complexity [50].

Patient communication may additionally improve substantially with 3D printed models. Visualization of pathology and planned reconstruction often facilitates patient understanding and informed consent [51].

Despite numerous advantages, limitations include production time, printing costs, sterilization challenges, and dependence on imaging quality [52]. Nevertheless, rapid advances in additive manufacturing continue to expand clinical applications.

Digital Implant Planning and Prosthetically Driven Reconstruction

Modern maxillofacial reconstruction increasingly emphasizes functional rehabilitation in addition to restoration of skeletal continuity and facial contour. Implant-supported prosthetic rehabilitation has therefore become a central objective of digital reconstructive workflows [53].

Prosthetically driven planning represents a major paradigm shift in reconstructive surgery. Instead of reconstructing bone solely according to anatomical shape, contemporary workflows begin with the desired prosthetic outcome and subsequently design skeletal reconstruction to support implant placement and occlusal function [54].

Digital implant planning combines CBCT imaging, intraoral scanning, virtual articulation, and CAD software to determine ideal implant position according to prosthetic requirements [55]. Implant placement may therefore be integrated directly into reconstructive planning.

Immediate implant placement during fibula free flap reconstruction has become increasingly feasible because of virtual surgical planning and CAD/CAM technologies [56]. Such approaches may significantly shorten rehabilitation time and improve patient quality of life.

Occlusal relationships may additionally be simulated digitally before surgery. Virtual articulation systems allow evaluation of mandibular movement, occlusal contacts, and prosthetic space requirements [57].

Surgical guides generated from implant planning software improve placement accuracy and reduce prosthetic complications [58]. Guided implant surgery is particularly important in reconstructed jaws where anatomy may be highly altered.

Digital workflows also facilitate immediate provisionalization and rapid prosthetic fabrication. Integration of implant planning with CAD/CAM prosthetics may substantially accelerate oral rehabilitation [59].

Several studies have demonstrated improved functional outcomes, speech, mastication, and patient satisfaction following digitally planned implant-supported reconstruction [60]. Nevertheless, adequate bone volume, vascularization, and soft tissue management remain essential prerequisites for long-term success.

Digital Workflow in Oncologic Reconstruction

Oncologic maxillofacial reconstruction represents one of the most complex applications of digital dentistry and virtual planning. Head and neck tumors often require extensive resection followed by challenging skeletal and soft tissue reconstruction [61].

Digital workflows significantly improve precision in tumor surgery by enabling accurate virtual planning of resection margins and reconstructive osteotomies [62]. Such precision may help preserve uninvolved tissue while maintaining oncologic safety.

Fibula free flap reconstruction has become one of the most important beneficiaries of virtual planning. Donor-site osteotomies may be planned digitally according to mandibular contour and prosthetic rehabilitation objectives [63].

Cutting guides generated through CAD/CAM technologies allow highly accurate transfer of virtual plans into surgery. This may improve graft adaptation, facial symmetry, and occlusal rehabilitation [64].

Reduction of ischemia time is another major advantage. Preoperative plate adaptation and osteotomy planning significantly shorten intraoperative reconstruction time [65]. Reduced ischemia duration may improve free flap survival and postoperative recovery.

Digital workflows also improve interdisciplinary communication among oncologic surgeons, reconstructive surgeons, prosthodontists, and engineers [66]. Collaborative planning is particularly important in complex head and neck reconstruction.

Speech, swallowing, mastication, and esthetics are increasingly integrated into digital planning objectives. Functional rehabilitation has therefore become more predictable and patient-centered [67].

Despite these advances, oncologic reconstruction remains challenging because tumor extent, soft tissue involvement, and intraoperative findings may occasionally differ from virtual predictions [68]. Surgeons must therefore remain adaptable despite detailed digital planning.

Navigation Surgery and Dynamic Guidance Systems

Surgical navigation systems represent another important component of digital maxillofacial surgery. Navigation technologies provide real-time intraoperative tracking of surgical instruments relative to patient anatomy and preoperative imaging datasets [69].

Navigation systems are particularly valuable in anatomically complex regions such as the orbit, skull base, temporomandibular joint, and craniofacial skeleton [70]. Real-time guidance may improve surgical accuracy and reduce injury risk to critical structures.

Dynamic implant navigation systems have additionally gained increasing popularity in implant dentistry and reconstructive surgery. Unlike static surgical guides, dynamic navigation allows real-time modification of drilling trajectories during surgery [71].

Augmented reality technologies are also emerging as promising tools in digital surgery. Virtual anatomical information may be superimposed onto the surgical field, potentially improving visualization and spatial orientation [72].

Robotic-assisted surgery represents another future direction in digital maxillofacial reconstruction. Robotic systems integrated with virtual planning and navigation may improve precision in highly delicate procedures [73].

Although navigation technologies improve surgical accuracy, limitations include high cost, technical complexity, registration errors, and learning curve requirements [74]. Nevertheless, continued advances are expected to increase accessibility and clinical integration.

Limitations and Challenges of Digital Workflow

Despite the remarkable advantages of digital dentistry and virtual surgical planning, several important limitations and challenges remain. One of the primary barriers is the high financial cost associated with advanced imaging systems, planning software, CAD/CAM production, navigation technologies, and 3D printing equipment [75].

Implementation of a fully digital workflow frequently requires substantial institutional investment and interdisciplinary infrastructure. Smaller clinical centers may therefore experience difficulty adopting advanced digital reconstruction systems [76].

Another important challenge involves the learning curve associated with digital technologies. Surgeons must acquire knowledge in imaging interpretation, segmentation, virtual planning, CAD/CAM integration, and navigation systems [77]. Inadequate training may compromise planning accuracy and surgical outcomes.

Digital workflows are also highly dependent on imaging quality and data acquisition accuracy. Motion artifacts, segmentation errors, and incorrect image fusion may propagate inaccuracies throughout the reconstructive process [78]. Even minor digital discrepancies may influence implant positioning, occlusion, or graft adaptation.

Production time represents another limitation. Although virtual planning improves intraoperative efficiency, fabrication of patient-specific guides and implants may require several days depending on manufacturing complexity [79]. Such delays may complicate urgent oncologic or trauma reconstruction.

Soft tissue prediction additionally remains challenging. While digital systems provide excellent skeletal visualization, prediction of postoperative soft tissue adaptation is still imperfect in many cases [80]. Facial esthetics and functional outcomes therefore continue to depend partly on surgical experience and biological variability.

Another important issue involves intraoperative flexibility. Patient-specific guides and implants are manufactured according to preoperative plans; however, unexpected anatomical findings during surgery may require deviation from the virtual workflow [81]. Surgeons must therefore maintain conventional surgical adaptability despite digital planning.

Regulatory and legal considerations are increasingly relevant as customized implants and digitally manufactured devices become more common. Validation of software accuracy, sterilization standards, and quality control protocols remain essential [82].

Data security and digital storage also represent growing concerns because patient imaging and planning files are frequently transferred among multiple digital platforms and collaborators [83].

Despite these limitations, continuous technological improvements and increasing clinical experience continue to improve reliability, efficiency, and accessibility of digital reconstruction workflows.

Future Perspectives

The future of digital dentistry and virtual surgical planning in maxillofacial reconstruction is expected to involve increasingly integrated, automated, and biologically driven workflows. Advances in artificial intelligence, machine learning, robotics, augmented reality, and regenerative medicine are likely to further transform reconstructive surgery [84].

Artificial intelligence-assisted segmentation and automated treatment planning may significantly reduce planning time and operator dependency [85]. AI systems may additionally help optimize osteotomy design, implant positioning, and reconstruction symmetry through predictive algorithms.

Augmented reality and mixed reality technologies are expected to become increasingly integrated into surgical navigation systems. Surgeons may eventually visualize virtual anatomical overlays directly within the operative field, improving precision and spatial orientation [86].

Robotic-assisted surgery represents another promising frontier. Integration of robotic systems with virtual planning and dynamic navigation may enable highly precise osteotomies and implant placement while reducing human error [87].

Bioprinting and regenerative medicine may further expand the reconstructive potential of digital workflows. Customized tissue-engineered bone and soft tissue constructs generated through digital design may eventually allow biologically functional craniofacial reconstruction [88].

Digital twin technology is another emerging concept involving creation of comprehensive virtual patient models integrating anatomy, biomechanics, occlusion, and physiological data [89]. Such systems may improve predictive simulation and individualized treatment planning.

Cloud-based collaboration platforms are additionally expected to enhance interdisciplinary communication among surgeons, prosthodontists, radiologists, and engineers worldwide [90]. Remote virtual planning and international collaboration may therefore become increasingly common.

Point-of-care manufacturing and in-house printing laboratories may reduce production delays and improve accessibility of customized surgical devices [91]. Faster workflows may be particularly beneficial in oncologic and trauma reconstruction.

Education and surgical training are also likely to evolve substantially. Virtual simulation systems, haptic feedback technologies, and immersive digital planning platforms may improve resident education and procedural rehearsal [92].

Although many technological innovations remain under active development, digital dentistry and virtual surgical planning are expected to become increasingly central components of precision-based maxillofacial reconstruction.

Conclusion

Digital dentistry and virtual surgical planning have fundamentally transformed contemporary maxillofacial reconstruction by introducing highly precise, patient-specific, and workflow-centered treatment approaches. Integration of advanced imaging, CAD/CAM technologies, intraoral scanning, 3D printing, navigation systems, and prosthetically driven planning has significantly improved reconstructive predictability and interdisciplinary collaboration.

Virtual surgical planning allows surgeons to simulate complex procedures preoperatively, evaluate anatomical limitations, optimize reconstruction strategies, and improve communication among surgical and prosthetic teams. Such workflows enhance surgical precision while reducing operative uncertainty and intraoperative adaptation.

CAD/CAM technologies and additive manufacturing have enabled fabrication of customized surgical guides, reconstruction plates, patient-specific implants, and anatomical models. These innovations improve reconstruction accuracy, reduce operative time, and facilitate prosthetically guided rehabilitation.

Digital workflows have become particularly valuable in oncologic reconstruction, fibula free flap surgery, implant-supported rehabilitation, craniofacial deformity correction, and trauma reconstruction. Integration of prosthetic planning into skeletal reconstruction has significantly improved functional outcomes involving mastication, speech, and esthetics.

Navigation systems, augmented reality technologies, and dynamic guidance platforms further expand the precision and safety of modern reconstructive surgery. Emerging developments involving artificial intelligence, robotic surgery, and bioprinting are expected to continue accelerating the evolution of digital reconstruction.

Despite substantial advantages, important challenges remain regarding cost, learning curve, manufacturing time, imaging accuracy, and intraoperative flexibility. Continued technological refinement and standardized training are therefore essential for broader clinical integration.

Overall, digital dentistry and virtual surgical planning represent a paradigm shift in maxillofacial reconstruction, moving the field toward increasingly personalized, minimally invasive, and precision-based surgical care.

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CHAPTER 2

Platelet Concentrates in Oral Surgery: PRF, CGF, and Beyond

Özge Özdal Zincir[1]

[1] Assoc. Prof. Dr., Istanbul University- Cerrahpasa, Faculty of Dentistry, Department of Oral and Maxillofacial Surgery ORCID: 0009-0000-9423-5764

Introduction

Regenerative approaches have become increasingly important in contemporary oral and maxillofacial surgery because of growing emphasis on minimally invasive treatment, accelerated healing, and biologically driven tissue regeneration. Among the most significant developments in regenerative dentistry is the use of autologous platelet concentrates, which have gained widespread popularity because of their ability to enhance wound healing and tissue repair through delivery of concentrated growth factors and cytokines [1].

Platelet concentrates are autologous blood-derived biomaterials obtained through centrifugation protocols designed to concentrate platelets, leukocytes, fibrin matrices, and bioactive molecules [2]. These preparations release multiple growth factors involved in angiogenesis, collagen synthesis, cell proliferation, osteogenesis, and tissue remodeling.

The first generation of platelet concentrates was platelet-rich plasma (PRP), introduced in the 1990s primarily in maxillofacial surgery and orthopedics [3]. PRP demonstrated promising regenerative potential but required anticoagulants and biochemical activation protocols, which complicated clinical use and affected fibrin architecture.

Subsequently, second-generation platelet concentrates such as platelet-rich fibrin (PRF) were developed to simplify preparation and improve biological properties [4]. PRF is characterized by a dense fibrin matrix containing platelets, leukocytes, cytokines, and circulating stem cells without the need for anticoagulants or exogenous thrombin activation.

Various modifications of PRF technology have since emerged, including leukocyte-rich PRF (L-PRF), advanced PRF (A-PRF), injectable PRF (i-PRF), titanium-prepared PRF (T-PRF), and concentrated growth factor (CGF) preparations [5]. These newer systems aim to optimize growth factor release, cellular content, fibrin structure, and regenerative potential.

Platelet concentrates have been investigated in numerous oral surgical procedures including tooth extraction, alveolar ridge preservation, sinus floor augmentation, implant surgery, periodontal regeneration, medication-related osteonecrosis management, temporomandibular joint disorders, and soft tissue healing [6].

One of the major advantages of platelet concentrates is their autologous origin, which minimizes immunological risk and disease transmission concerns [7]. Preparation protocols are relatively simple, cost-effective, and minimally invasive compared with synthetic biomaterials or recombinant growth factors.

Despite increasing popularity, controversies remain regarding standardization, centrifugation protocols, growth factor quantification, and

clinical effectiveness. Considerable variability exists among studies because of differences in preparation techniques, patient factors, and outcome measures [8].

This chapter reviews the biological basis, classification, preparation methods, mechanisms of action, clinical applications, limitations, and future perspectives of platelet concentrates in oral surgery, with particular focus on PRF, CGF, and emerging regenerative technologies.

Biological Basis of Platelet Concentrates

Platelets play a central role in tissue healing and regeneration through release of multiple growth factors, cytokines, chemokines, and bioactive proteins following activation [9]. Beyond their traditional role in hemostasis, platelets contribute significantly to inflammation, angiogenesis, extracellular matrix formation, and tissue remodeling.

When tissue injury occurs, activated platelets release alpha granule contents including platelet-derived growth factor (PDGF), transforming growth factor beta (TGF-β), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), insulin-like growth factor (IGF), and fibroblast growth factor (FGF) [10]. These molecules regulate cell migration, proliferation, angiogenesis, collagen synthesis, and osteoblastic differentiation.

Fibrin additionally plays a critical role in tissue regeneration. The fibrin matrix functions as a biological scaffold supporting cell migration, vascular ingrowth, and sustained growth factor release [11]. Dense fibrin architecture may therefore influence regenerative potential significantly.

Leukocytes present within platelet concentrates may further contribute to healing through immunological regulation and antimicrobial activity [12]. Neutrophils, monocytes, and lymphocytes release cytokines involved in inflammatory modulation and tissue remodeling.

Stem cells and circulating progenitor cells may also become incorporated into fibrin matrices during centrifugation [13]. These cells potentially contribute to regenerative processes through differentiation and paracrine signaling mechanisms.

Growth factor release kinetics vary among platelet concentrate systems depending on fibrin density, centrifugation speed, platelet concentration, and preparation protocol [14]. Sustained release profiles are generally considered beneficial for prolonged tissue stimulation.

Another important biological characteristic involves angiogenesis stimulation. Vascularization is essential for successful wound healing and bone regeneration because newly formed tissues require oxygen and nutrient supply [15]. VEGF and related mediators released from platelet concentrates play important roles in neovascularization.

Platelet concentrates may additionally modulate inflammatory responses and reduce postoperative discomfort. Cytokines and leukocyte-derived mediators influence both pro-inflammatory and anti-inflammatory pathways [16].

Understanding these biological mechanisms is essential for appropriate clinical application and optimization of regenerative outcomes in oral surgery.

Classification of Platelet Concentrates

Platelet concentrates may be broadly classified according to fibrin architecture, leukocyte content, preparation protocols, and biological characteristics. Dohan Ehrenfest and colleagues proposed one of the most widely accepted classification systems categorizing platelet concentrates into four major groups [17].

The first category includes pure platelet-rich plasma (P-PRP), characterized by high platelet concentration with minimal leukocyte content and liquid consistency before activation [18]. P-PRP requires anticoagulants during preparation and external activation using calcium chloride or thrombin.

Leukocyte-rich platelet-rich plasma (L-PRP) represents the second category and contains higher leukocyte concentrations in addition to platelets [19]. Leukocytes may contribute to antimicrobial activity and immunological regulation, although excessive inflammatory response remains a potential concern.

The third category includes pure platelet-rich fibrin (P-PRF), characterized by a dense fibrin matrix without leukocytes. These materials form solid fibrin membranes after centrifugation and do not require anticoagulants [20].

Leukocyte-rich platelet-rich fibrin (L-PRF) is the most commonly used platelet concentrate in oral surgery today. L-PRF contains platelets, leukocytes, cytokines, and a highly organized fibrin matrix capable of sustained growth factor release [21].

Subsequent technological modifications have produced additional platelet concentrate systems. Advanced PRF (A-PRF) was developed using lower centrifugation speeds to increase leukocyte and growth factor retention within the fibrin matrix [22].

Injectable PRF (i-PRF) is a liquid formulation prepared using very low-speed centrifugation protocols. Because of delayed polymerization, i-PRF may be mixed with graft materials or injected directly into tissues [23].

Titanium-prepared PRF (T-PRF) utilizes titanium tubes instead of glass tubes during centrifugation. Titanium is believed to induce improved fibrin polymerization and greater biocompatibility [24].

Concentrated growth factors (CGF) represent another advanced platelet concentrate generated using variable-speed centrifugation protocols. CGF reportedly demonstrates denser fibrin architecture and higher growth factor concentration than traditional PRF systems [25].

Although classification systems continue to evolve, standardization remains challenging because preparation protocols vary significantly among manufacturers and clinicians.

Platelet-Rich Fibrin (PRF)

Platelet-rich fibrin is currently one of the most extensively investigated platelet concentrates in oral and maxillofacial surgery. Introduced by Choukroun and colleagues in 2001, PRF was designed as a simplified second-generation platelet concentrate requiring no anticoagulants or biochemical additives [26].

PRF preparation is relatively straightforward. Venous blood is collected into sterile tubes and immediately centrifuged according to specific protocols. Delayed centrifugation may result in premature coagulation and compromise fibrin architecture [27].

After centrifugation, three layers are generally observed: red blood cells at the bottom, acellular plasma at the top, and the PRF clot located between them [28]. The PRF clot may subsequently be compressed into membranes or fragmented for grafting applications.

One of the major advantages of PRF is its dense fibrin matrix, which allows gradual release of growth factors over time. Studies have demonstrated sustained release of PDGF, TGF-β, VEGF, and other mediators for several days after preparation [29].

The fibrin network additionally functions as a scaffold supporting cell migration and angiogenesis. Leukocytes embedded within the matrix may contribute to immunological regulation and antimicrobial defense [30].

PRF has been investigated in numerous oral surgical procedures including extraction socket healing, ridge preservation, sinus augmentation, implant surgery, periodontal regeneration, and management of MRONJ [31].

Several studies have reported reduced postoperative pain, swelling, and trismus following PRF application after third molar surgery [32]. Improved soft tissue healing and accelerated epithelialization have additionally been observed.

In implant dentistry, PRF may enhance osseointegration and peri-implant soft tissue healing [33]. Combination of PRF with bone graft materials is particularly common in regenerative procedures.

Despite promising results, clinical evidence remains somewhat heterogeneous because of variability in centrifugation protocols, patient characteristics, and

outcome measures [34]. Nevertheless, PRF continues to represent one of the most accessible and biologically attractive regenerative adjuncts in oral surgery.

Advanced Platelet-Rich Fibrin (A-PRF)

Advanced platelet-rich fibrin was developed to optimize the biological characteristics of conventional PRF through modification of centrifugation parameters. The concept of low-speed centrifugation was introduced based on the hypothesis that lower centrifugal forces may preserve greater numbers of leukocytes, platelets, and regenerative cells within the fibrin matrix [35].

Compared with standard PRF protocols, A-PRF is generally prepared using lower revolutions per minute and longer centrifugation duration [36]. Reduced centrifugal force allows increased retention of neutrophils, monocytes, lymphocytes, and platelets in the upper portions of the fibrin clot.

Histological studies have demonstrated that A-PRF exhibits a more porous fibrin architecture and more homogeneous cellular distribution compared with conventional PRF [37]. These structural differences may influence growth factor release kinetics and cellular migration.

Several investigations have reported higher concentrations of growth factors such as VEGF, PDGF, and TGF-β in A-PRF preparations [38]. Sustained release of these mediators may improve angiogenesis and tissue regeneration.

A-PRF has been increasingly investigated in implant surgery, periodontal regeneration, sinus augmentation, and soft tissue healing [39]. Improved vascularization and enhanced soft tissue maturation have been reported in some clinical studies.

Another important application involves combination of A-PRF with particulate bone grafts. The fibrin matrix may function as a biological binder, improving graft stability and handling characteristics [40].

Despite promising biological findings, clinical superiority of A-PRF over conventional PRF remains incompletely established because of limited standardization and heterogeneity among studies [41]. Nevertheless, low-speed centrifugation concepts continue to gain popularity in regenerative oral surgery.

Injectable PRF (i-PRF)

Injectable platelet-rich fibrin represents another important evolution in platelet concentrate technology. Unlike conventional PRF membranes, i-PRF remains in liquid form for a short period before polymerization, allowing injection into tissues or mixing with graft materials [42].

i-PRF is prepared using very low-speed centrifugation protocols without anticoagulants. The reduced centrifugal force allows preservation of platelets, leukocytes, fibrinogen, and circulating stem cells within the plasma fraction [43].

One of the major advantages of i-PRF is its versatility. Liquid consistency permits direct injection into soft tissues, periodontal defects, temporomandibular joints, and extraction sockets [44]. i-PRF may additionally be combined with bone substitutes to form “sticky bone” grafts.

Sticky bone consists of particulate graft material agglutinated within fibrin-rich i-PRF matrices [45]. This composite structure improves graft stability, handling, and adaptation while potentially enhancing regenerative potential.

In implant dentistry, i-PRF has been investigated for enhancement of soft tissue healing, implant stability, and osseointegration [46]. Injectable formulations may additionally improve peri-implant mucosal quality and vascularization.

Several in vitro studies have demonstrated that i-PRF stimulates fibroblast proliferation, osteoblast differentiation, and angiogenesis [47]. Antimicrobial effects against periodontal pathogens and oral microorganisms have also been suggested.

Aesthetic applications are additionally emerging, particularly in facial rejuvenation and soft tissue augmentation procedures [48]. Growth factors and fibrin matrices may contribute to tissue remodeling and collagen synthesis.

Despite increasing popularity, standardization remains a major challenge because preparation protocols vary considerably among clinicians and manufacturers [49]. Optimal centrifugation parameters and clinical indications continue to be investigated.

Concentrated Growth Factors (CGF)

Concentrated growth factors represent another advanced platelet concentrate system introduced by Sacco in 2006 [50]. CGF is obtained through specialized variable-speed centrifugation protocols designed to produce denser fibrin matrices and higher concentrations of regenerative cells and growth factors.

Unlike traditional PRF systems using constant centrifugation speeds, CGF preparation involves alternating acceleration and deceleration phases [51]. This variable centrifugation process reportedly generates larger and denser fibrin structures.

Histological analyses have demonstrated that CGF contains abundant fibrin networks enriched with platelets, leukocytes, CD34-positive stem cells, and growth factors [52]. These characteristics may contribute to enhanced regenerative potential.

Several studies have suggested that CGF demonstrates prolonged release of growth factors compared with conventional PRF [53]. Increased fibrin density may additionally improve scaffold stability and cellular migration.

CGF has been investigated in extraction socket preservation, implant surgery, sinus floor augmentation, periodontal regeneration, and management of bone defects [54]. Improved soft tissue healing and enhanced bone formation have been reported in some clinical studies.

One of the most important applications of CGF involves sinus augmentation procedures. CGF membranes may protect the Schneiderian membrane and improve graft stabilization during sinus floor elevation [55].

CGF has additionally been combined with stem cell therapies and biomaterials in experimental regenerative protocols [56]. Synergistic interactions between fibrin matrices and mesenchymal stem cells may further enhance tissue regeneration.

Despite encouraging findings, evidence comparing CGF with PRF and other platelet concentrates remains limited. Standardized randomized clinical trials are still required to determine definitive clinical superiority [57].

Platelet Concentrates in Tooth Extraction and Socket Preservation

Tooth extraction remains one of the most common procedures in oral surgery and is frequently associated with postoperative pain, edema, alveolar osteitis, and alveolar ridge resorption [58]. Platelet concentrates have therefore been extensively investigated as adjunctive materials for improving extraction socket healing.

PRF application within extraction sockets may promote clot stability, angiogenesis, epithelialization, and bone regeneration [59]. Several studies have reported reduced postoperative pain and swelling following PRF placement after third molar surgery.

Alveolar osteitis prevention is another important indication. Enhanced fibrin stability and antimicrobial activity may reduce incidence of dry socket formation [60].

Socket preservation procedures have also benefited from platelet concentrate applications. Combination of PRF or CGF with particulate bone grafts may reduce postextraction ridge resorption and improve future implant site development [61].

Histological studies have demonstrated increased new bone formation and vascularization in sockets treated with platelet concentrates [62]. Accelerated soft tissue closure and improved patient comfort have additionally been observed.

PRF membranes may also function as biologic barriers in guided bone regeneration procedures [63]. Their autologous nature and growth factor content make them attractive alternatives or adjuncts to conventional collagen membranes.

Although many clinical studies support positive effects of platelet concentrates in extraction healing, heterogeneity in study design and preparation protocols continues to complicate interpretation of outcomes [64].

Platelet Concentrates in Implant Surgery

Implant dentistry represents one of the most important clinical application areas of platelet concentrates. Successful implant therapy depends on osseointegration, angiogenesis, and soft tissue healing, all of which may potentially be enhanced through growth factor delivery [65].

PRF and CGF have been used during implant placement, sinus augmentation, peri-implant defect management, ridge augmentation, and immediate implant procedures [66].

One proposed mechanism involves stimulation of osteoblastic activity and angiogenesis around implant surfaces [67]. Improved vascularization may accelerate early healing and bone maturation.

Platelet concentrates have also been applied around implants to improve peri-implant soft tissue quality and mucosal healing [68]. Enhanced keratinized tissue formation and soft tissue thickness have been reported in some studies.

Sinus floor augmentation procedures frequently incorporate PRF or CGF in combination with bone grafts [69]. Fibrin matrices may improve graft handling and stabilize particulate materials within the sinus cavity.

Another important application involves immediate implant placement in extraction sockets. Platelet concentrates may reduce the gap between implant surfaces and surrounding bone while promoting soft tissue closure [70].

Several investigations have additionally evaluated implant stability quotient (ISQ) values following PRF application. Some studies suggest improved early implant stability and accelerated osseointegration [71].

Nevertheless, evidence remains inconsistent regarding long-term implant survival benefits associated with platelet concentrate use [72]. Further standardized clinical studies are therefore required.

Platelet Concentrates in MRONJ and Complex Wound Healing

Medication-related osteonecrosis of the jaw represents one of the most challenging complications in oral surgery because of impaired bone remodeling, reduced vascularity, and delayed healing [73]. Platelet concentrates have therefore gained attention as potential adjuncts in MRONJ management.

PRF and CGF may improve vascularization, soft tissue healing, and inflammatory modulation in osteonecrotic lesions [74]. Autologous growth factors may additionally stimulate tissue regeneration in poorly healing wounds.

Several clinical reports have demonstrated favorable healing outcomes following surgical debridement combined with PRF application in MRONJ patients [75]. Reduced pain, improved mucosal closure, and decreased recurrence rates have been described.

Platelet concentrates have additionally been investigated in osteoradionecrosis, chronic extraction wounds, sinus perforation repair, and oroantral fistula management [76].

Complex medically compromised patients such as diabetics, smokers, and immunosuppressed individuals may also potentially benefit from biologically enhanced healing provided by platelet concentrates [77].

Although current evidence remains encouraging, larger randomized controlled studies are still necessary to establish standardized protocols and definitive clinical recommendations.

Limitations and Controversies

Despite the increasing popularity of platelet concentrates in oral surgery, several important limitations and controversies remain. One of the major challenges involves the lack of standardization among preparation protocols. Differences in centrifugation speed, centrifugation duration, tube material, blood volume, and processing time may significantly influence fibrin architecture, cellular composition, and growth factor release [78].

Considerable variability additionally exists among commercial systems and operator techniques. Consequently, results reported in clinical studies are often difficult to compare directly [79].

Another important limitation involves patient-related biological variability. Age, systemic diseases, smoking, medication use, hydration status, and hematological characteristics may all influence platelet concentration and regenerative potential [80].

The exact mechanisms underlying the clinical benefits of platelet concentrates also remain incompletely understood. Although growth factor release is considered a major regenerative mechanism, interactions among fibrin scaffolds, leukocytes, stem cells, cytokines, and local tissues are highly complex [81].

Clinical evidence remains heterogeneous for many applications. While some studies report significant improvements in bone regeneration, implant stability, and wound healing, others demonstrate limited or nonsignificant benefits compared with conventional treatment approaches [82].

Another controversial issue involves leukocyte content. Some investigators suggest that leukocytes contribute positively through antimicrobial activity and

immunological regulation, whereas others propose that excessive inflammatory response may impair healing [83].

Growth factor quantification and release kinetics are also difficult to standardize. Different laboratory methods and analytical techniques may produce variable results [84].

Platelet concentrates additionally possess relatively limited mechanical strength compared with synthetic membranes or biomaterials. Consequently, they are frequently used in combination with graft materials rather than as stand-alone regenerative scaffolds [85].

Storage and handling represent further limitations because platelet concentrates are autologous materials requiring immediate preparation and clinical use [86]. Unlike commercially available biomaterials, they cannot be stored long term.

Economic considerations may additionally influence clinical adoption. Although platelet concentrates are generally less expensive than recombinant growth factors or advanced biomaterials, specialized centrifugation systems and training are still required [87].

Despite these limitations, platelet concentrates continue to attract substantial clinical interest because of their autologous origin, favorable safety profile, and regenerative potential.

Future Perspectives

The future of platelet concentrates in oral surgery is expected to involve increasingly sophisticated biologically driven regenerative strategies. Ongoing advances in centrifugation technology, tissue engineering, biomaterials, stem cell therapy, and molecular biology are likely to expand both the effectiveness and clinical applications of platelet concentrates [88].

One important future direction involves optimization and standardization of preparation protocols. Improved understanding of centrifugation physics and cellular distribution may allow development of more reproducible and biologically efficient platelet concentrate systems [89].

Combination therapies are also expected to become increasingly important. Platelet concentrates may be integrated with stem cells, biomimetic scaffolds, growth factor delivery systems, and tissue-engineered constructs to enhance regenerative outcomes [90].

Injectable formulations and bioactive fibrin matrices may additionally facilitate minimally invasive regenerative procedures. Injectable PRF technologies may become particularly important in facial esthetics,

temporomandibular joint disorders, peri-implant soft tissue management, and minimally invasive bone regeneration [91].

Another promising area involves incorporation of platelet concentrates into three-dimensional bioprinting and digital regenerative workflows. Customized biologic scaffolds integrating platelet-derived growth factors and living cells may eventually allow highly individualized tissue reconstruction [92].

Molecular and genomic research may further improve understanding of the biological interactions underlying regenerative healing. Personalized regenerative protocols based on patient-specific biological characteristics may therefore become possible [93].

Artificial intelligence and digital image analysis may additionally contribute to objective evaluation of regenerative outcomes and optimization of treatment planning [94].

Long-term randomized clinical studies remain critically important for establishing evidence-based indications and standardized treatment guidelines. Greater methodological consistency is necessary to strengthen clinical recommendations [95].

As regenerative medicine continues to evolve, platelet concentrates are expected to remain among the most accessible and biologically attractive autologous materials in oral and maxillofacial surgery.

Conclusion

Platelet concentrates have become one of the most widely investigated regenerative adjuncts in contemporary oral and maxillofacial surgery. Through delivery of autologous growth factors, fibrin matrices, leukocytes, and bioactive mediators, these biomaterials contribute to tissue healing, angiogenesis, inflammation modulation, and bone regeneration.

Platelet-rich fibrin remains the most commonly used second-generation platelet concentrate because of its simplicity, low cost, autologous origin, and favorable biological characteristics. Subsequent developments including A-PRF, injectable PRF, T-PRF, and concentrated growth factors have further expanded the regenerative possibilities of platelet concentrate technology.

Clinical applications continue to broaden across extraction socket preservation, implant surgery, sinus augmentation, periodontal regeneration, MRONJ management, soft tissue healing, and complex reconstructive procedures. Many studies report reduced postoperative morbidity and improved regenerative healing associated with platelet concentrate use.

Nevertheless, important limitations remain regarding protocol standardization, biological variability, growth factor quantification, and

consistency of clinical evidence. Heterogeneity among studies continues to complicate direct comparison of outcomes and establishment of universal guidelines.

Future developments involving tissue engineering, stem cell integration, bioprinting, digital regenerative workflows, and precision medicine are likely to further enhance the role of platelet concentrates in oral surgery. Continued translational research and well-designed randomized clinical trials will remain essential for optimizing their clinical applications.

Overall, platelet concentrates represent a biologically promising and clinically versatile regenerative strategy that continues to shape the evolution of modern oral and maxillofacial surgery.

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CHAPTER 3

Stature Estimation from Long Bones and Related Segmental Measurements in the Turkish Population

Beyza Celik[1]

Seda Sertel Meyvaci[2]

[1] PhD Candidate, Department of Anatomy, Faculty of Medicine, Bolu Abant Izzet Baysal University, Bolu, Turkiye, 0000-0003-4401-8678

[2] Assoc. Prof. Dr., Department of Anatomy, Faculty of Medicine, Bolu Abant Izzet Baysal University, Bolu, Turkiye, 0000-0002-9450-145X

1. Introduction

Forensic anthropology is a fundamental scientific discipline that aims to establish a biological profile from unidentified human remains. This profile generally includes components such as age, sex, stature, and population ancestry. When these variables are evaluated together, the range of possible identities can be narrowed (Spradley, 2016). The identification process becomes more challenging in skeletonized or severely decomposed remains. In such cases, skeletal structures become the primary source of information because soft tissues are often not preserved. Therefore, changes occurring particularly in cases involving mass disasters, homicide, buried remains, and burned remains may affect long-bone measurements (Siddiqui et al., 2026; Barreiro et al., 2025; Kokati et al., 2025). Stature estimation is one of the most important components in the determination of the biological profile. When evaluated together with age, sex, and other skeletal findings, it increases the reliability of the identification process (Siddiqui et al., 2026). A clear relationship exists between human stature and skeletal elements. This relationship is particularly strong for the long bones of the extremities. Bones such as the femur, tibia, fibula, humerus, radius, and ulna are anatomical structures that directly contribute to overall stature (Kira et al., 2023).

Two main approaches are commonly used for stature estimation: the anatomical method and the mathematical method. The anatomical method is applied when the skeleton is largely complete and is based on summing the skeletal segments that contribute to stature (Brits et al., 2018). In this method, structures such as the skull, vertebral column, pelvis, lower-extremity bones, and foot height are evaluated together. Soft-tissue corrections are then added to estimate living stature. Although this method is theoretically robust, its applicability may be limited in forensic cases because the skeleton is often incomplete (Brits et al., 2018). The mathematical method, in contrast, is based on the statistical relationship between one or more bone lengths and stature. Linear regression equations are generally used for this purpose. Owing to its practical applicability in incomplete or fragmented remains, the mathematical method is widely used in forensic anthropology (Kamnikar et al., 2024).

Sex is one of the main variables affecting the accuracy of stature estimation equations. Long-bone lengths and body proportions may differ between male and female individuals. Therefore, sex-specific regression equations are frequently developed (Albanese et al., 2016; Bidmos & Brits, 2025; Sertel Meyvaci et al., 2025). Among the long bones, those of the lower extremity generally have greater predictive value. The femur and tibia are among the most frequently used bones because of their direct contribution to stature (Athani et al., 2016; Viqar & Khanna, 2018; Mahakkanukrauh et al., 2011). Upper-extremity bones are also important in forensic cases. The humerus, radius, and ulna may show lower

correlations with stature than lower-extremity bones in some circumstances. Nevertheless, they represent valuable sources for stature estimation when only upper-extremity remains are available (Ahmed, 2013; Albanese et al., 2016; Ismail et al., 2018). Hand and foot measurements may also be considered complementary parameters. In particular, hand length, finger lengths, foot length, and foot width have been reported to be associated with stature (Jee & Yun, 2015; Sen et al., 2014; Kanchan et al., 2008; Chikhalkar et al., 2010).

From an anatomical perspective, stature results from the cumulative contribution of multiple skeletal segments. Cranial height, the vertebral column, pelvis, lower-extremity bones, and foot segments all contribute to overall stature. However, in forensic cases, preservation of all these structures together is not always possible (Siddiqui et al., 2026). Therefore, the ability to use long bones independently represents an important advantage. Even a fragment of a femur, tibia, or humerus may contribute to the establishment of the biological profile. Mathematical methods become particularly advantageous in cases involving incomplete or fragmented remains (Kokati et al., 2025). A study that developed a model for estimating total femoral length from fragmented dry femora in the Turkish population also demonstrated that preserved proximal or distal fragments may be used to estimate total femoral length and thereby contribute to subsequent stature estimation when the complete femur is unavailable (Sertel Meyvaci et al., 2025).

Population differences are another fundamental consideration in stature estimation. Body proportions, genetic background, nutrition, environmental conditions, socioeconomic status, and secular changes may vary among populations. Consequently, an equation developed for one population may not provide the same level of accuracy when applied to another population (Albanese et al., 2016; Radoinova et al., 2002; Bidmos & Brits, 2025). This emphasizes the importance of population-specific standards in forensic anthropology. Equations that are not appropriate for a given population may overestimate or underestimate stature. Such inaccurate estimates may, in turn, lead to the erroneous exclusion of potential identities during the identification process (Spradley, 2016). Stature estimation also provides a link between osteological and clinical anatomical knowledge. The proximal and distal ends of bones, measurement landmarks, and segment lengths must be accurately defined. Anatomical landmarks should be standardized to minimize measurement errors (Kira et al., 2023). In recent years, radiological methods have increasingly been used in stature estimation studies. Conventional radiographs, computed tomography (CT) images, and three-dimensional reconstructions enable bones to be measured digitally. These approaches allow non-invasive assessment in both living individuals and postmortem examinations (Kira et al., 2023).

Studies on stature estimation in the Turkish population demonstrate that long bones and their related segments have been comprehensively evaluated using different samples and measurement methods. In addition to long bones such as the femur, tibia, fibula, humerus, radius, and ulna, anthropometric measurements of the hands, feet, and extremity segments have been investigated, and various estimation models have been developed using dry bones, cadavers, living individuals, and radiological images. Overall, the findings indicate that lower-extremity long bones generally show stronger relationships with stature, whereas upper-extremity bones and hand and foot measurements may provide complementary information in cases involving incomplete or fragmented remains. The effects of sex-specific body proportions, measurement methods, and sample characteristics on predictive performance further support the importance of developing models specific to the Turkish population.

Accordingly, this chapter reviews studies on stature estimation in the Turkish population based on upper- and lower-extremity long bones and their related segmental and anthropometric measurements, organized according to anatomical regions, and evaluates the measurement methods, estimation approaches, and findings from a forensic anthropological perspective.

2. Stature Estimation from Upper-Extremity Long Bones and Related Segmental Measurements in the Turkish Population

2.1. Humerus

The humerus is one of the important anatomical structures evaluated in stature estimation because it is the longest bone of the upper extremity. Although lower-extremity long bones generally show stronger relationships with stature, the humerus provides an important alternative when bones such as the femur or tibia are unavailable. Particularly in fragmented or incomplete skeletal remains, humeral length may serve as a supportive parameter for estimating an individual's stature (Ismail et al., 2018). Studies conducted in the Turkish population indicate that the humerus can be evaluated using both conventional osteometric and radiological methods.

Acikgoz et al. evaluated the lengths of the femur, tibia, fibula, humerus, radius, and ulna for stature estimation using radiographic measurements in an adult Anatolian population. According to their findings, the highest correlation with stature in both females and males was observed for the maximum length of the right humerus (Acikgoz et al., 2021).

Uzun et al. investigated stature estimation from anthropometric measurements of the upper extremity. The study evaluated upper-extremity segmental measurements that may be directly related to humeral length and examined their relationship with stature. The authors reported that anthropometric parameters of the upper extremity could be used for stature estimation and that segmental

measurements may provide practical data, particularly when long bones are unavailable or cannot be measured directly. This study is valuable in demonstrating the complementary role of upper-extremity segments, including those related to the humerus, in stature estimation (Uzun et al., 2019).

Akman et al. evaluated morphometric measurements of different humeral segments. Although the study did not directly aim to estimate stature, it is important in demonstrating the measurability and morphometric characteristics of different humeral segments. In fragmented remains where the complete humerus is not available, segmental morphometry may contribute to the estimation of total bone length or other biological characteristics of the individual (Akman et al., 2006).

Iris and Celbis evaluated stature estimation from humeral length in the Turkish population. Separate regression equations were developed for females and males based on humeral length. The standard error of stature estimation was reported as ±3.81 cm in males and ±3.05 cm in females. The authors stated that the estimation errors obtained from humeral length were comparable to those reported in studies involving other long bones. This study represents one of the principal sources that can be directly used for stature estimation from the humerus in the Turkish population (Iris & Celbis, 2003).

Tetiker et al. conducted a morphometric study to estimate total humeral length from dry humeral segments in an Anatolian population. Different segmental measurements were evaluated in 124 dry humeri. Although the study did not directly estimate the stature of individuals, it contributes to the determination of total humeral length in fragmented humeral remains. In this respect, it provides an indirect but important intermediate step for stature estimation from the humerus (Tetiker et al., 2023).

2.2. Radius and Ulna

The radius is the laterally located long bone of the forearm and, together with the ulna, represents one of the principal structures used in upper-extremity-based stature estimation. Maximum radial length is an important measurement reflecting upper-extremity segment length. The ulna is the medially located long bone of the forearm, and its maximum length, measured between the olecranon and the ulnar styloid process, is one of the parameters frequently used in stature estimation. The ulna also has an important place in anthropometric studies because it is relatively suitable for external measurement, particularly in living individuals (Sarma et al., 2022). In the Turkish population, the radius and ulna have generally been evaluated together.

Celbis and Agritmis investigated stature estimation and sex determination from radial and ulnar lengths in a Turkish cadaveric sample. In the study, the lengths of the radius and ulna were measured, and their relationships with the

individuals' stature and sex were evaluated. The authors reported that radial and ulnar lengths are reliable parameters that can be used for stature estimation in Turkish cadaveric samples (Celbis & Agritmis, 2006).

Uzun et al. also evaluated forearm segments in their study on stature estimation from upper-extremity anthropometric measurements. The representation of the radius through upper-extremity segmental measurements rather than direct bone length is important from clinical and practical anthropometric perspectives. The study demonstrated that externally obtainable measurements, such as forearm length, are associated with an individual's stature. Therefore, the radius and forearm segments may be considered together in stature estimation (Uzun et al., 2019).

Duyar and Pelin evaluated the need for a population-specific formula for stature estimation from ulnar length. The relationship between ulnar length and stature was examined, and the applicability of equations developed in different populations to Turkish individuals was discussed. The authors emphasized that ulnar length can be used for stature estimation; however, population-specific regression equations are required to obtain reliable results in the Turkish population (Duyar & Pelin, 2010).

Sargin et al. examined stature estimation from ulnar and tibial lengths in a cadaver-based study. Group-specific regression equations were developed, and the ulna was evaluated as a principal bone representing the upper extremity. The combined use of ulnar and tibial lengths demonstrates the contribution of both upper- and lower-extremity bones to combined stature estimation (Sargin et al., 2012).

2.3. Hand Bones and Measurements

Although hand bones are not used as directly as conventional long bones, they can provide complementary information in forensic anthropological cases. In particular, measurements such as metacarpal dimensions, phalangeal lengths, hand length, and hand width are among the anthropometric parameters associated with an individual's stature. Studies conducted in the Turkish population on hand length, metacarpal measurements, and finger lengths demonstrate the supportive role of the hand region in stature estimation.

Sagir evaluated stature estimation from radiographic measurements of the metacarpal bones in the Turkish population. In the study, metacarpal lengths were measured on hand radiographs, and the relationship between these measurements and individual stature was investigated. The author demonstrated that metacarpal measurements may contribute to stature estimation, particularly in cases where long bones are unavailable. This study represents one of the principal sources directly addressing stature estimation from hand bones in the Turkish population (Sagir, 2006).

Ozaslan et al. investigated the predictive role of hand and foot dimensions in stature estimation. Measurements including hand length, hand width, foot length, and foot width were evaluated. The findings indicated that the combined use of hand and foot dimensions may contribute to stature estimation. This study is particularly important in demonstrating that hand measurements can be incorporated into multivariable prediction models together with distal lower-extremity measurements rather than being used solely as isolated parameters (Ozaslan et al., 2012).

Mutluay et al. evaluated stature estimation from second- and fourth-digit lengths in young adults. The relationship between finger lengths and individual stature was investigated. Although second- and fourth-digit lengths represent smaller anatomical parameters compared with conventional long-bone measurements, they demonstrate the complementary role of the hand region in stature estimation. This study may be considered particularly relevant in cases where only hand or finger remains are available, as it provides potentially useful supplementary data (Mutluay et al., 2019).

Uzun et al. evaluated hand and upper-extremity segments together in their investigation of stature estimation from upper-extremity anthropometric measurements. The study demonstrated that stature could be estimated using hand length and other external measurements of the upper extremity. Hand measurements provide practical data in living individuals because they are easy to obtain and non-invasive. Therefore, the hand region may be considered a complementary anatomical area supporting long-bone-based stature estimation (Uzun et al., 2019).

3. Stature Estimation from Lower-Extremity Long Bones and Related Segmental Measurements in the Turkish Population

3.1. Femur

The femur is one of the most frequently evaluated anatomical structures in stature estimation because it is the longest and strongest bone in the human body. The strong relationship between lower-extremity length and overall body stature makes the femur an important parameter in forensic anthropological identification studies (Zhang et al., 2021; Imai et al., 2020). Studies based on femoral length in the Turkish population are also important because they emphasize the need for population-specific regression equations.

Sertel Meyvaci et al. evaluated the relationship between segmental lower-extremity measurements and stature using orthoroentgenographic images in a Turkish adult sample. Pelvic segment height and thigh segment length were identified as the most significant predictors of stature in males, whereas thigh segment length, leg segment length, and foot segment height were the most significant predictors in females. Total lower-extremity segment length and

trochanteric segment length showed strong relationships with stature in both sexes. This study demonstrated that lower-extremity segments contribute differently to stature estimation according to sex and anatomical region and that radiographic segmental measurements may have forensic utility for stature estimation from fragmented body parts in the Turkish population (Sertel Meyvaci et al., 2026).

Karacam et al. evaluated stature estimation from femoral length in autopsy cases from the Turkish population. In the study, stature and femoral length were measured in 95 individuals who underwent autopsy at the Istanbul Council of Forensic Medicine. The authors aimed to provide data for stature estimation in the Turkish population by examining the relationship between femoral length and stature. The study is important in demonstrating that the femur is one of the principal long bones that can be used for stature estimation in the Turkish population (Karacam et al., 2005).

Ekizoglu et al. evaluated stature estimation from lower-extremity long bones using CT images in a contemporary Turkish population. The study included 102 adults aged 20–87 years with known stature, and the lengths of the femur, tibia, and fibula were measured using three-dimensional CT reconstructions. In univariate models, fibular length showed the highest predictive accuracy in the pooled sample. When evaluated by sex, femoral length produced the lowest estimation error in males, whereas fibular length produced the lowest estimation error in females. The study demonstrated that CT-based measurements of the femur, tibia, and fibula can be reliably used for stature estimation in the Turkish population and highlighted the importance of population-specific regression equations (Ekizoglu et al., 2026).

Acikgoz et al. evaluated the lengths of the femur, tibia, fibula, humerus, radius, and ulna for stature estimation from radiographic measurements in an adult Anatolian population. Radiographic images of 167 healthy individuals were examined; both upper- and lower-extremity bones were measured in 88 individuals, whereas only lower-extremity bones were measured in 79 individuals. The femur, tibia, and fibula were included among the lower-extremity measurements, while the humerus, radius, and ulna were included among the upper-extremity measurements in the stature estimation models. The highest correlation with stature in both females and males was observed for the maximum length of the right humerus (Acikgoz et al., 2021).

Gunay et al. anthropometrically evaluated the lengths of upper- and lower-extremity bones in the Turkish population. The primary aim of the study was not to develop a direct stature estimation equation, but rather to provide population-specific long-bone data for Turkish individuals. The femur was among the lower-extremity bones evaluated in the study. Therefore, the study may be regarded as

a supportive source for the morphometric characteristics of femoral length in the Turkish population (Gunay et al., 2001).

Sertel Meyvaci et al. examined the relationship between the pelvis and lower-extremity long bones using orthoroentgenographic images of 200 Turkish individuals. The maximum right and left lengths of the femur, tibia, and fibula were measured, and their relationships with pelvic measurements were evaluated. The findings showed that the femur and tibia had stronger relationships with pelvic measurements than the fibula. This study demonstrated that pelvic measurements may assist in estimating lower-extremity bone lengths in cases involving incomplete or commingled skeletal remains (Sertel Meyvaci et al., 2025).

3.2. Tibia

The tibia is one of the most frequently used long bones for stature estimation in the lower extremity after the femur. Because it is one of the principal weight-bearing bones and contributes substantially to total lower-extremity length, a strong relationship is expected between tibial length and stature. Particularly in forensic cases where the femur is unavailable or damaged, the tibia represents an important alternative for stature estimation (Mahakkanukrauh et al., 2011). Studies on tibial length in the Turkish population indicate that this bone can be used through both conventional anthropometric and radiological approaches.

Günay et al. conducted one of the principal studies in the Turkish literature on stature estimation from tibial length. The relationship between tibial length and individual stature was evaluated, with the aim of obtaining population-specific data for the Turkish population. The authors emphasized that tibial length can be used for stature estimation, but that population-specific equations are required to achieve reliable estimates. This study may be regarded as one of the main sources for tibia-based stature estimation in the Turkish population (Gunay et al., 1996).

Pelin and Duyar compared different methods used for stature estimation from tibial length. In the study, stature values estimated from tibial length were compared with actual stature in 110 healthy Turkish male individuals. The authors evaluated the extent to which equations developed for different populations in the literature produced appropriate results when applied to Turkish individuals. The findings demonstrated that the direct application of equations derived from other populations may introduce error and that a population-specific approach is necessary for the Turkish population (Pelin & Duyar, 2003).

In a separate study, Duyar and Pelin investigated how classification of individuals into different stature groups affected stature estimation based on tibial length. This approach is important because it addresses whether the same regression equation provides the same level of accuracy in short-, medium-, and tall-stature groups. The study demonstrated that the relationship between tibial

length and stature should be evaluated not only at the overall population level but also with consideration of stature groups (Duyar & Pelin, 2003).

Sargın et al. evaluated stature estimation from ulnar and tibial lengths in a cadaveric sample. Group-specific regression equations were developed, and the role of tibial length in cadaver-based stature estimation was examined. The tibia was considered one of the principal lower-extremity parameters showing a strong relationship with stature. The cadaveric nature of the sample makes this study particularly relevant to forensic anthropological applications (Sargin et al., 2012).

3.3. Fibula

Although the fibula has been studied less frequently than the femur and tibia, it has complementary value among the lower-extremity long bones for stature estimation. Anatomically, its role in weight bearing is less prominent than that of the tibia; however, because it forms part of the lower-extremity length, fibular measurements may also be associated with stature. Particularly when the tibia or femur is damaged, the fibula may be considered an alternative or supportive bone. Although the number of studies directly addressing the fibula in the Turkish population is limited, thesis-based and radiological studies provide relevant data for this topic.

Ekizoglu et al. evaluated the fibula together with the femur and tibia using CT-based measurements in a contemporary Turkish population. Fibular length was measured on three-dimensional reconstructions and included in sex-specific regression models. According to the findings, fibular length was among the best-performing parameters in females and was notable for its low estimation error. This result indicates that the fibula should not be overlooked in stature estimation, particularly in specific subgroups (Ekizoglu et al., 2026).

Marangoz developed stature estimation equations from upper-arm, forearm, thigh, and calf lengths measured using kinanthropometric methods. The study was conducted on a total of 101 university students, including 48 males and 53 females. In males, stature showed a strong positive relationship with upper-arm and forearm lengths, a moderate positive relationship with thigh length, and a weak positive relationship with calf length. In females, stature showed a strong positive relationship with upper-arm length and a moderate positive relationship with calf length. Although direct bone measurements or radiographic measurements were not used, this study may be considered a complementary source because it demonstrates that upper- and lower-extremity segment lengths can contribute to stature estimation in the Turkish population (Marangoz, 2024).

3.4. Foot Bones and Measurements

Although foot measurements are not as conventional a method for stature estimation as long-bone measurements, they may provide important information in forensic anthropology, particularly in cases involving fragmented remains,

footprints, and shoeprints. Foot length, foot width, foot index, and shoe measurements are considered among the anthropometric parameters associated with an individual's stature. Because the foot bones represent the distal segment of the lower extremity, they can provide supportive measurements for stature estimation (Chikhalkar et al., 2010; Kanchan et al., 2008).

Ozden et al. developed equations for estimating stature and sex from foot and shoe measurements. The study included 569 individuals, comprising 294 males and 275 females, in whom right and left shoe size, maximum foot length, foot width, shoe length, and shoe width were measured. Significant relationships were observed between stature and particularly foot and shoe lengths, and length measurements were reported to have greater predictive value than width measurements. The authors indicated that foot and shoe dimensions could be used as supportive parameters for both stature estimation and sex determination. This study is important in demonstrating that crime-scene evidence such as footprints or shoeprints may contribute to stature estimation in the Turkish population (Ozden et al., 2005).

Sanli et al. investigated the relationship among hand length, foot length, and stature in adult Turkish individuals living in Adana. A total of 155 adults, including 80 males and 75 females, were evaluated. Hand length and foot length were considered independent variables, whereas stature was treated as the dependent variable, and multiple linear regression models were developed. The findings demonstrated that foot length, together with hand length, may serve as a practical anthropometric parameter for stature estimation (Sanli et al., 2005).

Zeybek et al. evaluated stature and sex estimation from foot measurements. Equations for stature estimation and sex determination were developed using foot length and other foot measurements. The findings showed that foot measurements could provide high accuracy in sex estimation and could also be used for stature estimation. In particular, foot length was identified as one of the parameters strongly associated with stature (Zeybek et al., 2008).

Atamturk and Duyar examined age-related factors in the relationship between foot measurements, living stature, and body weight. The study is important not only in terms of stature estimation from foot measurements, but also in addressing the influence of age on this relationship. Age-related changes in stature may affect the interpretation of equations based on foot measurements. Therefore, this study supports the consideration of age when estimating stature from foot dimensions (Atamturk & Duyar, 2008).

Atamturk et al. investigated the applicability of stature estimation equations derived from foot measurements of living individuals to cadavers. The study included 136 cadavers aged 18–50 years, comprising 76 males and 60 females, in whom stature, foot length, and foot width were measured. When equations developed from Turkish and non-Turkish populations were applied to the

cadaveric sample, the formulas generally overestimated stature. Equations developed for the Turkish population produced mean overestimations ranging from +2 to +9 cm, whereas equations derived from other populations produced overestimations ranging from +6 to +12 cm. The authors therefore emphasized that stature estimation equations based on foot measurements obtained from living individuals should be applied cautiously in forensic cadaveric cases (Atamturk et al., 2021).

Parlak et al. evaluated sex and stature estimation from anthropometric foot measurements in an Eastern Turkish sample using linear analyses and an artificial neural network approach. The study was conducted on 134 medical students, including 69 males and 65 females. By comparing conventional linear methods with artificial neural network models, the authors demonstrated that foot measurements could also be analyzed using modern statistical approaches. This study is important in providing a contemporary, artificial intelligence–supported perspective on stature estimation from foot measurements in the Turkish population (Parlak et al., 2024).

CONCLUSION

In conclusion, long bones represent reliable and applicable anatomical sources for stature estimation in the Turkish population. Among the lower-extremity bones, particularly the femur and tibia stand out because of their strong contribution to overall stature, whereas the fibula and foot measurements may provide complementary information in incomplete or fragmented remains. In the upper extremity, the humerus, radius, and ulna offer important alternatives when lower-extremity bones are unavailable, while hand bones and hand measurements may be considered supportive anthropometric parameters. Studies conducted in the Turkish population have demonstrated significant relationships between stature and various bone and segmental measurements and have shown that the use of population-specific regression equations can improve estimation accuracy. Nevertheless, because sex, age, measurement method, sample characteristics, and the statistical model used may influence the results, each study should be interpreted within the context of its own limitations.

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CHAPTER 4

The Dental Functional Loss (DFL) Index: A Data-Informed Model for Assessing Dentition-Derived Masticatory Structural Loss

Necmi Namal[1]

1

WHY IS A DFL APPROACH NEEDED?

Oral rehabilitation aims not only to replace missing teeth or restore damaged dental structures but also to preserve a dentition capable of supporting mastication, occlusal stability, and other essential oral functions. Contemporary concepts of oral health therefore extend beyond the absence of disease and increasingly emphasize functional capacity, quality of life, and maintenance of oral function throughout life (1-6). Consequently, assessment of function has become an important objective in both clinical dentistry and oral health research (7-12).

Despite this broader perspective, epidemiological assessment of dental health continues to rely predominantly on indices that quantify disease experience rather than its potential masticatory structural consequences. The Decayed, Missing and Filled Teeth (DMFT) index remains the international standard for measuring cumulative dental caries experience because of its simplicity, reproducibility, and value for population surveillance (2,3,13). However, DMFT treats each affected tooth as an equivalent unit regardless of anatomical position, biomechanical characteristics, or functional role. It also does not differentiate the extent to which different levels of structural damage may compromise the functional contribution of an affected tooth. Consequently, identical DMFT scores may represent substantially different patterns of dentition-related functional burden.

Recognition of the importance of functional dentition has led to complementary approaches such as the Functional and Sound Teeth (FS-T) index, the Functional Tooth Unit (FTU) concept, and the Shortened Dental Arch (SDA) concept (14-19). These approaches represented important advances by emphasizing retained teeth, occlusal support, and functional dentition rather than cumulative disease experience alone. Nevertheless, they primarily characterize preserved dentition or functional support and were not specifically designed to quantify masticatory structural loss by simultaneously considering which tooth is affected and the extent to which its structure and functional potential have been compromised.

This distinction is important because the human dentition is functionally heterogeneous. Incisors, canines, premolars, and molars differ in morphology, periodontal support, occlusal loading, contact characteristics, and their contributions during different mandibular functions (7–10,18,20). Incisors are particularly important for incision and protrusive function; canines participate in tearing and frequently contribute to lateral guidance; premolars provide transitional crushing and masticatory support; and molars carry a major proportion of posterior occlusal load and grinding function. Thus, loss or severe structural compromise of the same number of teeth need not represent an equivalent reduction in the functional potential of the dentition.

Tooth identity, however, represents only one dimension of this problem. The extent of structural involvement is also relevant. A small lesion that does not materially alter the functional morphology of a tooth cannot reasonably be assumed to represent the same degree of masticatory structural loss as extensive coronal destruction or complete tooth loss. Similarly, the presence of a restoration does not necessarily imply functional loss when the tooth remains structurally and functionally integrated within the dentition. A function-oriented framework should therefore distinguish both the relative functional contribution of the affected tooth and the severity of masticatory structural compromise.

Our previous work has explored complementary weighting approaches for dental health assessment, demonstrating that conventional disease counts and severity-sensitive or function-oriented measures need not classify the same dentition identically (14,15). These observations support a broader methodological principle: cumulative disease experience, structural severity, retained dentition, and functional potential represent related but non-equivalent dimensions of oral health. An index intended to characterize dentition-derived functional loss should therefore not infer functional consequences solely from the number of affected teeth.

A further distinction is essential. Structural characteristics of the dentition cannot provide a direct measurement of overall oral function. Actual masticatory performance is influenced not only by dental status but also by neuromuscular function, occlusal relationships, saliva, pain, adaptation, prosthetic rehabilitation, and other individual factors. Patient-perceived functional impact additionally incorporates psychosocial and quality-of-life dimensions. Accordingly, a dentition-based index should not be interpreted as a substitute for objective masticatory-performance testing or oral-health-related quality-of-life instruments. Rather, it may quantify a specific component of oral functional health: the masticatory structural loss attributable to the condition and functional importance of individual teeth. This distinction is consistent with conceptual frameworks that treat oral health as multidimensional rather than reducible to a single clinical measure (22).

To address this methodological gap, this chapter presents the Dental Functional Loss (DFL) Index as a two-dimensional, data-informed framework. The proposed model integrates tooth-specific functional coefficients, informed by anatomical and biomechanical evidence, with a graded masticatory structural severity component reflecting the extent to which an individual tooth is compromised. The contribution of each affected tooth is therefore determined jointly by which tooth is affected and how extensively its masticatory structural integrity has been lost.

THE CONCEPTUAL FOUNDATION OF DFL

Disease experience, retained dentition, and masticatory structural loss

Dental indices must be interpreted according to the construct they measure. DMFT primarily represents cumulative caries experience, FS-T represents retained sound or successfully restored dentition, and FTU/SDA approaches describe functional tooth presence and occlusal support in different ways (2-8). DFL is not designed to replace these measures. It addresses a different question: how much masticatory structural loss is present in the current dentition when the relative contribution of different tooth groups is considered?

Accordingly, DMFT, FS-T, and DFL may classify the same individual differently without creating a theoretical contradiction. A person with extensive past caries experience but successful restoration and preserved masticatory morphology may have a high DMFT but a relatively low DFL. Conversely, loss of a small number of strategically important posterior teeth may generate a higher DFL despite a modest conventional disease count.

Why should all teeth not receive equal weight?

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A further distinction is essential. Structural characteristics of the dentition cannot provide a direct measurement of overall oral function. Actual masticatory performance is influenced not only by dental status but also by neuromuscular function, occlusal relationships, saliva, pain, adaptation, prosthetic rehabilitation, and other individual factors. Patient-perceived functional impact additionally

incorporates psychosocial and quality-of-life dimensions. Accordingly, a dentition-based index should not be interpreted as a substitute for objective masticatory-performance testing or oral-health-related quality-of-life instruments. Rather, it may quantify a specific component of oral functional health: the masticatory structural loss attributable to the condition and functional importance of individual teeth.

The core DFL assumption is therefore not FC_incisor = FC_canine = FC_premolar = FC_molar, but FC_incisor < FC_canine < FC_premolar < FC_molar. The functional coefficient (FC) is not a physical constant or a direct measure of the amount of chewing performed by a tooth. It is a relative weighting parameter representing the contribution of a tooth group to the masticatory structural capacity of the dentition.

FROM A CONCEPTUAL APPROACH TO DATA-INFORMED COEFFICIENTS

DFL originated from the conceptual premise that tooth groups are not equivalent in their masticatory structural contribution. During model development, the purpose of coefficient calibration was not to confirm predetermined numbers, but to determine what relative weighting structure was supported by independent quantitative anatomical and biomechanical evidence. This distinction is central to the present formulation of the index.

BIOLOGICAL BASIS OF THE TOOTH-GROUP COEFFICIENTS

Why is a single biological variable insufficient?

The functional-coefficient calibration was based primarily on two objective tooth-specific domains for which comparable quantitative measurements were available across the dentition: occlusal-force distribution and periodontal/root support area.

For each domain, tooth-specific values were grouped into incisors, canines, premolars, and molars and expressed relative to the mean value for incisors, which served as the reference category. Because the domains represent different biological dimensions and are measured on different physical scales, normalized ratios rather than raw measurements were used for coefficient development.

Equal-weight geometric aggregation was used as the primary method for combining the normalized biomechanical and anatomical domains. This approach was selected to avoid allowing a single domain with a larger numerical range to dominate the composite estimate. Alternative aggregation and weighting assumptions were examined conceptually as sensitivity analyses to assess the stability of the resulting tooth-group hierarchy.

The primary calibration therefore used two tooth-specific quantitative domains: occlusal-force distribution and periodontal/root-support area. Effective

occlusal contact area was used as a third, independent domain to examine the posterior premolar-molar relationship.

Occlusal-force distribution

Occlusal force is not distributed equally along the dental arch. Tooth-specific measurements show a marked anterior-to-posterior increase, with molars carrying a substantial proportion of total load. The mandibular half-arch force distributions reported by Hejne (15) were grouped by tooth class and normalized to the mean incisor value.

Table 1. Relative occlusal-force calibration used in the DFL model

Tooth group	Relative occlusal-force ratio
Incisor	1.00
Canine	1.19
Premolar	2.98
Molar	7.04

This distribution demonstrates a clear anterior-posterior gradient in masticatory load bearing. Occlusal force alone, however, was not used as the DFL coefficient because it would incompletely represent the structural support characteristics of teeth, particularly the canine.

Periodontal/root-support area

The ability of a tooth to withstand masticatory loading depends not only on the magnitude of force applied to it but also on the supporting root-periodontal interface. Published mandibular root-surface-area measurements from Oda (16) were grouped by tooth class and normalized to the mean incisor value.

Table 2. Relative periodontal/root-support calibration used in the DFL model

Tooth group	Relative root-support ratio
Incisor	1.00
Canine	1.60
Premolar	1.24
Molar	2.28

The canine is particularly informative in this domain: its static occlusal-force share is modest, but its periodontal support area is relatively large. This illustrates why no single biomechanical variable was considered sufficient for coefficient development.

Construction of the primary composite coefficient

Because occlusal-force distribution and periodontal support are measured in different physical units and represent different biological domains, raw values were not added directly. Each domain was normalized to the incisor reference,

and an equal-weight geometric mean was used to avoid dominance by the numerically larger domain (21).

Table 3. Primary composite calibration of DFL tooth-group coefficients

Tooth group	Force ratio	Root-support ratio	Geometric mean	Scaled estimate (incisor = 3)
Incisor	1.00	1.00	1.00	3.00
Canine	1.19	1.60	1.38	4.14
Premolar	2.98	1.24	1.92	5.76
Molar	7.04	2.28	4.01	12.02

These primary estimates strongly supported practical coefficients of 3 for incisors, 4 for canines, and 6 for premolars. Because the molar estimate was more sensitive to biomechanical assumptions, the molar coefficient was examined against an independent posterior calibration domain.

INDEPENDENT POSTERIOR CALIBRATION OF THE MOLAR COEFFICIENT

Effective occlusal contact area is particularly relevant in the posterior dentition. In the classic measurements of Yurkstas and Manly (17), the effective contact contribution of the first premolar, second premolar, first molar, and second molar was approximately 8.1%, 12.9%, 36.7%, and 27.9%, respectively. Averaging within tooth groups gives 10.5 for premolars and 32.3 for molars, corresponding to a molar-to-premolar ratio of approximately 3.08.

Table 4. Independent posterior calibration of the molar coefficient

Domain	Molar/premolar ratio
Occlusal-force distribution	2.37
Periodontal/root support	1.84
Effective occlusal contact area	3.08
Geometric aggregation of the three ratios	2.37
Premolar coefficient × aggregated ratio	6 × 2.37 = 14.22
Practical molar coefficient	14

The proposed DFL coefficients are therefore 3, 4, 6, and 14 for incisors, canines, premolars, and molars, respectively. These values are provisional, data-informed relative weighting parameters. They are not universal biomechanical constants and may be recalibrated if future clinical evidence supports different values.

THE SECOND DIMENSION OF DFL: SEVERITY OF STRUCTURAL LOSS

Masticatory Structural Severity Classification

To distinguish the presence of disease from the extent of functional compromise, each tooth is assigned a masticatory structural severity score ranging from 0 to 3.

The severity hierarchy was designed to avoid treating a minor lesion, extensive coronal destruction, and complete tooth loss as equivalent events. The 0–3 structure represents an ordinal progression of masticatory structural involvement and requires future clinical calibration against independently measured functional outcomes.

Importantly, the presence of a restoration does not automatically constitute functional loss. A restored tooth that retains functional morphology and integration within the dentition may therefore receive an S0 classification, whereas structural compromise associated with extensive disease or treatment may receive a higher severity classification according to its remaining functional integrity.

Table 5. Operational S0-S3 recording criteria for masticatory structural loss

Code	S value	Clinical recording criterion
S0	0	No clinically meaningful loss of masticatory coronal structure. Sound teeth and restorations that preserve relevant morphology may be recorded here.
S1	1	Localized coronal structural loss; most of the masticatory surface/edge and expected structural contribution are preserved.
S2	2	Substantial loss of the functional crown or masticatory morphology, while the tooth remains present and retains partial masticatory structural potential.
S3	3	Complete or near-complete loss of masticatory coronal structure incompatible with meaningful contribution, or absence of the tooth.

The S0-S3 hierarchy is ordinal. The current transformation of S1 and S2 to one-third and two-thirds of the maximum severity is an initial linear scoring rule; it must not be interpreted as evidence that exactly 33% or 67% of true masticatory function has been lost.

CLINICAL ASSESSMENT AND FIELD RECORDING OF DFL

DFL is intended to be recordable by standardized intraoral clinical examination, in a manner compatible with epidemiological field use. Routine panoramic or intraoral radiography is not required for calculation of the score. This approach is consistent with the practical orientation of WHO basic oral-health survey methods, in which standardized clinical examination is the principal field procedure (1).

Recommended basic examination conditions

The examination should use adequate illumination, a plane mouth mirror, appropriate infection-control procedures, and a systematic tooth-by-tooth sequence. Third molars are excluded from the standard 28-tooth DFL calculation. The examiner records only the S category for each tooth; the tooth-group functional coefficient is assigned from tooth identity during scoring.

Clinical decision rule: coronal structural loss is the primary criterion

DFL severity is not a classification of caries depth. The decisive question is the degree to which clinically observable coronal structure relevant to mastication has been lost. A radiographically deep lesion should therefore not automatically receive a higher S category if clinically relevant masticatory morphology remains largely preserved. Conversely, obvious coronal destruction may be classified clinically without requiring radiographic confirmation of lesion depth. Etiology does not determine the score; the current structural state does.

Role of radiography

Radiographs may provide useful supplementary information when already available for clinical reasons, but they are not a mandatory component of routine DFL field scoring. Requiring radiography would reduce feasibility in population surveys and would shift the construct toward lesion depth rather than observable masticatory structural loss.

Proposed DFL Oral Assessment Form

For epidemiological use, a simple FDI-based recording form is proposed. Each of the 28 evaluated teeth receives a single S code (0-3). The examiner does not need to enter the coefficient; software or a scoring sheet can assign FC automatically from tooth number. The form should be regarded as a proposed research instrument until inter- and intra-examiner reliability and operational thresholds have been validated.

Table 6A. DFL Oral Assessment Form - Upper right

Tooth	**17**	**16**	**15**	**14**	**13**	**12**	**11**
S code							

Table 6B. DFL Oral Assessment Form - Upper left

Tooth	**21**	**22**	**23**	**24**	**25**	**26**	**27**
S code							

Table 6C. DFL Oral Assessment Form - Lower left

Tooth	**37**	**36**	**35**	**34**	**33**	**32**	**31**
S code							

Table 6D. DFL Oral Assessment Form - Lower right

Tooth	**41**	**42**	**43**	**44**	**45**	**46**	**47**
S code							

BUILDING THE DFL SCORE

For each tooth, the DFL contribution is obtained by multiplying the tooth-group functional coefficient by the normalized severity weight.

Table 7. Tooth-level DFL calculation rule

Component	**Definition**
Tooth-level equation	DFL_i = FC_i × (S_i / 3)
FC_i	Functional coefficient assigned to the tooth group
S_i	Masticatory structural-loss severity score, 0-3
Individual DFL	Sum of all tooth-level DFL contributions

Table 8. Tooth-specific DFL contribution by severity level

Tooth group	**FC**	**S0**	**S1**	**S2**	**S3**
Incisor	3	0	1.00	2.00	3.00
Canine	4	0	1.33	2.67	4.00
Premolar	6	0	2.00	4.00	6.00
Molar	14	0	4.67	9.33	14.00

SCORE RANGE, NORMALIZATION, AND INTERPRETATION

Table 9. Theoretical maximum DFL score for a 28-tooth dentition

Tooth group	**Number of teeth**	**FC**	**Maximum contribution**
Incisors	8	3	24
Canines	4	4	16
Premolars	8	6	48
Molars	8	14	112
Total			200

The absolute theoretical range is therefore 0-200. For comparison across individuals or populations, the score may also be expressed as a percentage of the theoretical maximum: DFL (%) = (DFL / 200) × 100. A value of 100% does not mean that 100% of total oral function has been lost; it denotes only the theoretical condition in which all 28 evaluated teeth are classified at S3 within the DFL construct.

THE MEASUREMENT BOUNDARIES OF DFL

DFL Does Not Directly Measure Oral Function

An essential boundary of the DFL construct is that structural dental status cannot be translated directly into total oral function. Mastication is a complex biological process influenced by dentition, occlusal relationships, neuromuscular coordination, salivary function, pain, sensory feedback, adaptation, prosthetic rehabilitation, and systemic or neurological conditions. An individual may therefore have a complete dentition yet demonstrate impaired mastication, while another individual with substantial tooth loss may partially compensate through adaptation or rehabilitation.

Accordingly, DFL should not be interpreted as a substitute for objective measurements of masticatory performance or efficiency. Such tests provide direct information about realized chewing performance and should serve as important external criteria in future validation studies. The conceptual role of DFL is different: it estimates the component of potential functional loss attributable specifically to the structural condition and relative functional importance of the dentition.

The same distinction applies to patient-reported outcomes. Oral-health-related quality-of-life instruments capture dimensions that extend well beyond dental biomechanics, including pain, appearance, psychosocial impact, social interaction, and perceived functional limitation. For example, loss of an anterior tooth may have a profound aesthetic or psychosocial impact even when its biomechanical weighting is lower than that of a molar. DFL does not attempt to assign numerical values to such individual perceptions and should therefore be regarded as complementary to, rather than a replacement for, patient-reported outcome measures.

In concise terms, DFL does not answer the question “How well does this person chew?” It addresses the narrower question “How much masticatory structural potential has been lost as a consequence of the structural state of the natural dentition?”

THE PLACE OF DFL AMONG EXISTING ORAL-HEALTH MEASURES

Relationship to Existing Oral Health Indices

The DFL framework is not intended to replace DMFT, FS-T, FTU, SDA, or other established measures. These approaches quantify different dimensions of oral health and may therefore provide complementary information.

Conceptually, DMFT describes cumulative disease experience; FS-T and related measures characterize retained or potentially functional dentition; DFL estimates dentition-derived masticatory structural loss. A patient may consequently have substantial cumulative disease experience but relatively limited current DFL if affected teeth have been successfully restored and remain structurally and functionally integrated. Conversely, loss or severe destruction of strategically important posterior teeth may produce substantial DFL despite a comparatively modest number of affected teeth.

This distinction may help explain why disease-oriented and function-oriented indices do not necessarily classify individuals identically. Rather than representing competing measures, these indices may be considered different views of a multidimensional oral health state.

Table 10. Complementary measurement dimensions

Measure	**Primary construct**
DMFT	Cumulative caries experience
FS-T	Retained sound and functionally restorable dentition
FTU/SDA	Functional occlusal support and dentition configuration
DFL	Weighted dentition-derived masticatory structural loss
Objective chewing tests	Realized masticatory performance/efficiency
OHRQoL measures	Patient-perceived functional, esthetic, and psychosocial outcomes

READING DFL THROUGH CLINICAL EXAMPLES

The model provides two forms of differentiation that simple tooth counts cannot represent: tooth identity and severity of structural loss.

Table 11. Illustrative DFL scenarios

Scenario	Calculation	DFL
Complete loss of two incisors	2 × 3	6
Complete loss of two molars	2 × 14	28
Molar with S1 loss	14 × 1/3	4.67
Molar with S2 loss	14 × 2/3	9.33
Molar with S3 loss	14 × 3/3	14

These examples must not be interpreted as statements of total value to the patient. An anterior tooth may have far greater esthetic, phonetic, or psychosocial importance than its DFL coefficient suggests. DFL weights only the defined masticatory structural construct.

HOW SHOULD THE S0-S3 SEVERITY SYSTEM BE INTERPRETED?

Incorporating the Severity of Masticatory Structural Compromise

Tooth identity alone is insufficient to characterize masticatory structural loss. A minor lesion that does not materially alter functional morphology cannot reasonably be assumed to have the same consequence as extensive coronal destruction or complete tooth loss. This consideration led to the second major component of the revised model: a graded masticatory structural severity scale.

The proposed S0–S3 classification distinguishes no meaningful masticatory structural loss, limited involvement, substantial involvement, and complete or near-complete loss. Consequently, DFL does not treat the presence of disease as synonymous with complete loss of the functional contribution of the affected tooth. A minor lesion may therefore produce little or no DFL-defined loss, whereas extensive destruction of the same tooth generates a progressively greater contribution to the score.

The present 0–3 structure should, however, be interpreted cautiously. Normalization of S1 and S2 as one-third and two-thirds of the maximum severity provides a transparent and reproducible initial scoring rule, but it does not imply that these categories have already been demonstrated to correspond to exactly 33% and 67% reductions in clinical function. Whether the relationship between structural compromise and functional loss is linear requires empirical investigation. Future studies should therefore compare alternative severity functions and determine whether clinical outcomes support linear, non-linear, or tooth-specific severity weighting.

Restored Teeth and Functional Loss

The treatment of restored teeth represents another important distinction between DFL and conventional disease-experience measures. In DMFT, a filled tooth remains part of cumulative caries experience. In a masticatory structural framework, however, the existence of a restoration does not necessarily indicate

current functional loss. A successfully restored tooth with preserved morphology, occlusal integration, and functional contribution may therefore receive an S0 classification.

Conversely, extensive restoration, severe loss of coronal structure, endodontic treatment, altered occlusal morphology, or other clinical conditions should not automatically be assigned a predetermined DFL value solely because they are present. Their relevance to DFL depends on the extent to which masticatory structural integrity is actually compromised. This distinction preserves the conceptual separation between disease history, treatment history, and current dentition-derived masticatory structural loss.

ROBUSTNESS OF MODEL DECISIONS

The coefficient hierarchy remained stable across alternative normalization and aggregation assumptions considered during model development. Exact numerical values are more sensitive than the ordering itself. The scientific claim of DFL is therefore not that 3, 4, 6, and 14 are immutable natural constants, but that independent biological evidence consistently supports a non-equivalent hierarchy of incisor < canine < premolar < molar, and that 3-4-6-14 is a practical first data-informed scaling of that relationship.

POTENTIAL APPLICATIONS OF DFL

Potential Applications and Validation Strategy

If validated, DFL may provide a standardized framework for comparing patterns of masticatory structural dental loss between individuals and populations and for investigating relationships between dental status and independently measured functional outcomes. Potential applications include oral epidemiology, oral rehabilitation research, prosthodontics, longitudinal assessment of dentition, and evaluation of changes following treatment.

Clinical use for treatment prioritization should, however, be considered premature. A higher DFL contribution does not by itself establish that one tooth should receive treatment before another, because treatment decisions also depend on prognosis, pain, infection, patient preferences, aesthetics, periodontal status, restorability, cost, and broader clinical circumstances. The immediate role of DFL is therefore primarily as a measurement framework rather than a prescriptive treatment algorithm.

Validation should proceed in several stages. First, reproducibility of the S0–S3 severity classification should be assessed using inter- and intra-examiner reliability. Second, construct validity should be examined by testing whether DFL behaves as expected across clinically different patterns of tooth loss and structural damage. Third, criterion-related validity should be investigated against objective measures of masticatory performance, occlusal contact, and other relevant

functional outcomes. Fourth, relationships with patient-reported oral function and oral-health-related quality of life should be examined without assuming that these constructs are equivalent. Finally, population-based studies should evaluate distribution, interpretability, responsiveness, and potential ceiling or floor effects.

If responsiveness is demonstrated, longitudinal use may allow changes from S0 to S1, S2, or S3 to be detected before tooth absence alone would capture progression. Conversely, successful treatment that restores relevant structural morphology could theoretically reduce the DFL burden. Such use requires empirical validation of change sensitivity.

WHAT SHOULD DFL NOT BE USED FOR?

DFL is not a treatment-prioritization algorithm. A higher molar coefficient does not mean that a molar should always be treated before an incisor. Pain, infection, prognosis, restorability, periodontal condition, esthetic need, patient preference, cost, systemic health, and treatment risk remain independent components of clinical decision making. The initial role of DFL should therefore be measurement and research, not prescriptive treatment ranking.

STRENGTHS OF THE MODEL

The principal strength of the DFL framework is its explicit separation of tooth-specific functional importance from severity of masticatory structural compromise. The coefficient-development procedure is transparent and reproducible, and sensitivity analysis indicates that the overall functional hierarchy is robust to reasonable alternative aggregation assumptions. The model also avoids automatically equating disease, restoration, and tooth loss with identical functional consequences.

Additional strengths are mathematical transparency, traceability of each score to a specific tooth and severity category, the ability to distinguish treatment history from current structural state, and a theoretical maximum of 200 that permits straightforward percentage normalization. Equally important, the model explicitly states what it does not measure.

LIMITATIONS OF THE MODEL

Several limitations remain. First, DFL is currently a theoretical and evidence-informed model and has not undergone clinical validation. Second, the provisional functional coefficients depend on published anatomical and biomechanical measurements obtained from different study populations and methodologies; they should therefore be recalibrated when sufficiently comprehensive tooth-specific datasets become available. Third, the S0–S3 severity categories require operational clinical criteria and formal reliability testing. Fourth, the present linear transformation of severity scores remains an

initial mathematical assumption rather than an empirically established dose-response relationship.

Furthermore, DFL does not directly incorporate occlusal scheme, antagonist status, periodontal disease, prosthetic replacement, neuromuscular function, salivary dysfunction, pain, adaptation, aesthetics, or psychosocial consequences. Some of these variables may eventually prove useful as modifiers, whereas others are likely to remain conceptually outside the DFL construct. Expanding the model should therefore be guided by empirical validation rather than by an attempt to incorporate every determinant of oral function into a single index.

Coefficients derived from different data sources

Occlusal-force, periodontal-support, and effective-contact data were not obtained from the same individuals using a single standardized protocol. Combining normalized values from different populations and methods is appropriate for model development but cannot be regarded as final biomechanical calibration.

Provisional nature of the coefficients

The 3-4-6-14 system represents a data-informed starting set. More comprehensive tooth-specific datasets may support recalibration.

Clinical operationalization of S0-S3

The conceptual categories require explicit examiner training, photographic examples, and inter- and intra-examiner reliability testing before routine field use.

Assumption of a linear severity function

The one-third and two-thirds transformations for S1 and S2 are initial scoring assumptions. The true relation between structural loss and masticatory outcome may be nonlinear.

Antagonist status

The current formula does not directly incorporate the presence or condition of the opposing tooth. This may affect realized occlusal participation and should be investigated empirically before any antagonist modifier is added.

Occlusal scheme

Canine guidance, group function, and other occlusal arrangements vary between individuals. Current coefficients are population-level tooth-group weights rather than individualized occlusal models.

Periodontal disease

Anatomical root-support area contributes to coefficient development, but an individual tooth’s current periodontal disease severity is not separately scored.

Prosthetic rehabilitation

Implants, fixed prostheses, and removable prostheses may improve realized chewing performance, but the present DFL framework describes structural loss in the natural dentition. Prosthetic compensation requires separate validation.

Variables outside the DFL construct

Neuromuscular function, saliva, pain, adaptation, systemic or neurological disease, esthetics, and psychosocial outcomes remain outside the direct measurement domain.

FUTURE RESEARCH AND VALIDATION ROADMAP

Reliability

Inter- and intra-examiner reproducibility of S0-S3 recording should be established first.

Construct validity

Predefined hypotheses should test whether posterior loss and greater structural destruction produce the expected DFL patterns.

Criterion-related validity

DFL should be compared with objective masticatory-performance measures. A perfect correlation is neither expected nor required because realized performance is influenced by many non-dental variables.

Validation against occlusal contact

Associations between total/regional DFL and regional effective occlusal contact area may be particularly informative for posterior calibration.

Patient-reported outcomes

Associations with perceived chewing difficulty and OHRQoL should be studied while recognizing that these measures represent broader constructs.

Population behavior

Distribution, age patterns, socioeconomic gradients, floor/ceiling effects, interpretability, and sensitivity to different tooth-loss patterns should be assessed across populations.

Responsiveness

Longitudinal and pre/post-treatment studies should determine whether DFL detects clinically meaningful structural change over time.

OVERALL PERSPECTIVE: A TESTABLE MEASUREMENT FRAMEWORK

Overall Interpretation

The DFL framework moves beyond simple tooth counting by integrating two biologically relevant dimensions: the relative functional contribution of the affected tooth and the severity of its masticatory structural compromise. In doing so, it provides a standardized estimate of dentition-derived masticatory structural loss while explicitly avoiding the claim that structural dental findings alone constitute a direct measurement of total oral function.

The proposed model should therefore be viewed as a testable measurement framework rather than a completed clinical instrument. Its value will depend on whether the provisional coefficients and severity structure demonstrate reproducibility, construct validity, criterion-related validity, and responsiveness in independent clinical and population-based studies. If these properties are confirmed, DFL may complement established disease-based, retained-dentition, objective functional, and patient-reported measures and contribute to a more multidimensional assessment of oral health.

The central innovation is therefore not the numerical coefficients alone. It is the integration of two questions within one transparent framework: which tooth is affected, and how much of its masticatory structural contribution has been lost? Future recalibration of coefficients or severity weights would not invalidate this principle; it would represent the expected empirical development of the model.

FINAL PERSPECTIVE: THE SCIENTIFIC POSITION AND FUTURE OF DFL

The Dental Functional Loss (DFL) Index introduces a data-informed, two-dimensional framework for estimating dentition-derived masticatory structural loss. By integrating the relative functional contribution of individual tooth groups with the severity of masticatory structural compromise, the model extends conventional tooth-count–based assessment beyond the assumption that all affected teeth and all degrees of dental damage represent equivalent loss.

The DFL Index is intended to complement rather than replace established oral health measures. Whereas disease-based indices characterize cumulative disease experience and retained-dentition measures describe preserved dental status, DFL addresses a distinct dimension: the weighted masticatory structural loss attributable to the identity and condition of individual teeth. Importantly, DFL does not directly measure masticatory performance, patient-perceived oral function, or overall oral functional status.

The present study establishes the theoretical and methodological basis of the revised framework and proposes provisional, data-informed tooth-group

coefficients together with a graded masticatory structural severity system. These components require independent evaluation of reproducibility, biomechanical and clinical validity, and responsiveness. If validated against objective functional measures and patient-centred outcomes, the DFL Index may provide a complementary tool for oral rehabilitation research, dental epidemiology, and multidimensional assessment of dentition-related functional health.

DFL should therefore be regarded not as a finished clinical instrument, but as an empirically testable and recalibratable framework for quantifying the masticatory significance of tooth loss and structural dental damage within the natural dentition.

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CHAPTER 5

Current Approaches to Optical Imaging of Neuronal Activity

Ömer Yusuf İpek[1]

[1] MD, PhD., Kırşehir Ahi Evran University, Faculty of Medicine, Department of Physiology, ORCID: 0000-0003-4538-7141

1. INTRODUCTION

Understanding how the nervous system works depends on the ability to monitor the electrical activity of neurons with sufficient temporal and spatial resolution. For more than a century, the principal tool for this task has been electrophysiology: intracellular and patch-clamp recordings measure the membrane potential of a single neuron with microsecond precision, and extracellular multi-electrode arrays can register the firing patterns of hundreds of neurons simultaneously. Electrophysiology, however, has two structural limitations. First, the identity and spatial arrangement of the recorded cells are often uncertain; an extracellular electrode cannot directly report which neuron generated a given signal. Second, intracellular recordings are restricted to a handful of cells at a time, and fine structures such as dendritic branches or axonal terminals remain technically very difficult to access.

Optical methods have developed to fill this gap. The underlying idea is simple: a molecule that converts a physical quantity linked to neuronal activity (intracellular calcium concentration, membrane potential, neurotransmitter release) into a change in fluorescence is introduced into the cell, and this fluorescence is recorded with a microscope. In this way, the activity of hundreds or even thousands of cells can be followed within the same field of view, with the identity and location of each cell known. Over the past two decades, genetically encoded indicators have made this approach targetable to specific cell types (Grienberger, Giovannucci, Zeiger, & Portera-Cailliau, 2022); miniaturized microscopes have enabled recordings lasting weeks in freely moving animals (Aharoni & Hoogland, 2019); and kilohertz-rate imaging systems have brought optical recordings close to the time scale of electrophysiology (Nguyen et al., 2025).

This chapter reviews current approaches to the optical imaging of neuronal activity within a comparative framework. It begins with the basic principles of calcium imaging and the available indicator options, then turns to imaging platforms, the trade-offs among speed, resolution and sensitivity, the relationship between calcium imaging and direct voltage imaging, and finally data analysis. The aim is not to provide a detailed protocol for any single technique, but to offer an up-to-date map of which experimental question is best answered with which tool.

2. FUNDAMENTALS OF CALCIUM IMAGING

2.1. Why Calcium Is a Proxy for Activity

In a resting neuron, the cytosolic free calcium (Ca^{2+}) concentration is about 50–100 nM, whereas the extracellular concentration is in the millimolar range, tens of thousands of times higher. During an action potential, the opening of voltage-gated calcium channels transiently raises cytosolic calcium ten- to a

hundred-fold (Grienberger & Konnerth, 2012); synaptic inputs generate dendritic calcium transients through NMDA receptors and release from intracellular stores. This wide dynamic range makes calcium a naturally amplified reporter of neuronal activity: whereas a change of one hundred millivolts in membrane potential is difficult to measure optically, the accompanying rise in calcium is readily visualized with an appropriate fluorescent probe.

The relationship, however, is indirect and not always linear. The calcium signal outlasts the action potential by tens to hundreds of milliseconds, a duration set by intracellular buffering, pumps and the binding kinetics of the indicator itself. Calcium imaging therefore delivers a temporally smoothed projection of the firing pattern whose amplitude may saturate. This limitation is revisited later in the chapter in the context of spike inference and voltage imaging.

2.2. Synthetic Calcium Indicators

The first generation of calcium imaging relied on the BAPTA-derived chelators developed by Tsien and colleagues in the 1980s. Ratiometric dyes such as Fura-2 and Indo-1 allow the absolute calcium concentration to be estimated from the ratio of fluorescence at two wavelengths (Grynkiewicz, Poenie, & Tsien, 1985). The acetoxymethyl (AM) ester forms of these dyes cross the cell membrane passively, are cleaved by intracellular esterases and become trapped in the cytosol, so that hundreds of cells in a tissue slice can be loaded in a single incubation step (Stosiek, Garaschuk, Holthoff, & Konnerth, 2003; Tsien, 1981). Single-wavelength dyes such as Fluo-4, Oregon Green BAPTA-1 and, more recently, Cal-520 do not permit ratiometric measurement but produce far larger fluorescence changes ($\Delta F/F$) and are therefore widely preferred for detecting transients evoked by action potentials (Tada, Takeuchi, Hashizume, Kitamura, & Kano, 2014).

The advantages of synthetic dyes are fast kinetics, independence from genetic manipulation and immediate applicability in acute preparations. Their disadvantages are the absence of cell-type selectivity, gradual leakage and compartmentalization into organelles, and unsuitability for long-term (days to weeks) recordings. A point requiring particular care with AM ester loading is the tendency of the dye to accumulate more strongly in glial cells than in neurons; slow, large-amplitude signals observed in slices may therefore reflect astrocytic calcium waves rather than neuronal activity. Nevertheless, when recordings are made at sufficiently high frame rates, fast and small-amplitude (1–3%) signals of neuronal origin can also be resolved in hippocampal slices loaded with Fluo-4 AM (İpek, Abbas, Sajidy, & Canepari, 2025). This finding shows that synthetic dyes remain a valuable tool in acute slice work.

2.3. Genetically Encoded Calcium Indicators

Genetically encoded calcium indicators (GECIs) are built by fusing a fluorescent protein to a calcium-binding domain, most often calmodulin. The most widely used family, GCaMP, is based on a circularly permuted green fluorescent protein inserted between calmodulin and a target peptide: when calcium binds, calmodulin wraps around the peptide, the resulting conformational change is transmitted to the fluorescent protein and its fluorescence increases (Nakai, Ohkura, & Imoto, 2001). Early GCaMPs saw limited use because of low brightness and slow kinetics; GCaMP3 improved protein stability, and the GCaMP6 family raised sensitivity enough to make reliable in vivo detection of single action potentials possible for the first time (Chen et al., 2013; Tian et al., 2009). The jGCaMP7 variants offered subtypes optimized for different applications (fast, sensitive, bright) (Dana et al., 2019), and the XCaMP series added sensors in additional colors with improved linearity (Inoue et al., 2019).

The most recent leap in the field is the jGCaMP8 series, obtained by redesigning the calmodulin–peptide interface and replacing the conventional RS20 peptide with one derived from endothelial nitric oxide synthase; the resulting sensors have half-rise times in the millisecond range and markedly higher sensitivity than previous generations (Zhang et al., 2023). The three principal variants (8f, 8m and 8s) occupy different positions along the speed–sensitivity axis: 8f is the fastest, 8s the most sensitive and 8m a balanced option. This kinetic improvement has, for the first time, brought the temporal resolution of calcium imaging close to the level needed to distinguish individual action potentials in spike trains of up to 50 Hz (Zhang & Looger, 2024). Recent ground-truth analyses have shown that the most linear variants, 8s and 8m, reliably detect isolated action potentials in cortical pyramidal neurons, whereas 8f and earlier generations do not reach this level (Rupprecht, Rózsa, Fang, Svoboda, & Helmchen, 2026).

GECIs in colors other than green are also developing rapidly. Red sensors such as jRGECO1a and jRCaMP1 (Dana et al., 2016) allow imaging deeper in tissue and simultaneous use with a green sensor or with an optogenetic actuator excited by blue light. Cell-type-specific promoters, Cre-dependent viral vectors and transgenic lines allow GECI expression to be confined to a defined neuronal population, a degree of selectivity that synthetic dyes have never offered. GECIs have their costs, however: viral expression takes weeks, overexpression can disturb intracellular calcium buffering, and signal linearity may be lost, particularly at high firing rates. The in vitro properties of a sensor do not always predict its in vivo behavior; characterization of the chosen indicator in the investigator's own experimental system is therefore indispensable (Zhang & Looger, 2024). Table 1 summarizes the principal features of the two indicator classes.

Table 1. Comparison of synthetic and genetically encoded calcium indicators

Feature	Synthetic dyes (Fura-2, Fluo-4, Cal-520)	GECIs (GCaMP6/7/8, jRGECO1a)
Loading	AM ester incubation or microinjection (minutes to 1 h)	Viral vector, transgenic line or electroporation (days to weeks)
Cell-type selectivity	None; tendency toward glial loading	High, via promoter and Cre systems
Kinetics	Generally fast (especially low-affinity derivatives)	Generation-dependent; millisecond range with jGCaMP8
Chronic recording	Not suitable (hours)	Suitable (weeks to months)
Absolute concentration	Possible with ratiometric dyes	Limited (mostly ΔF/F)
Color options	Broad (UV to red)	Green dominant; red and near-infrared emerging
Typical use	Acute slices, cell culture, fast signal analysis	In vivo, chronic, cell-type-specific population studies

Compiled by the author from Grienberger and Konnerth (2012), Tada et al. (2014) and Zhang and Looger (2024).

3. IMAGING PLATFORMS

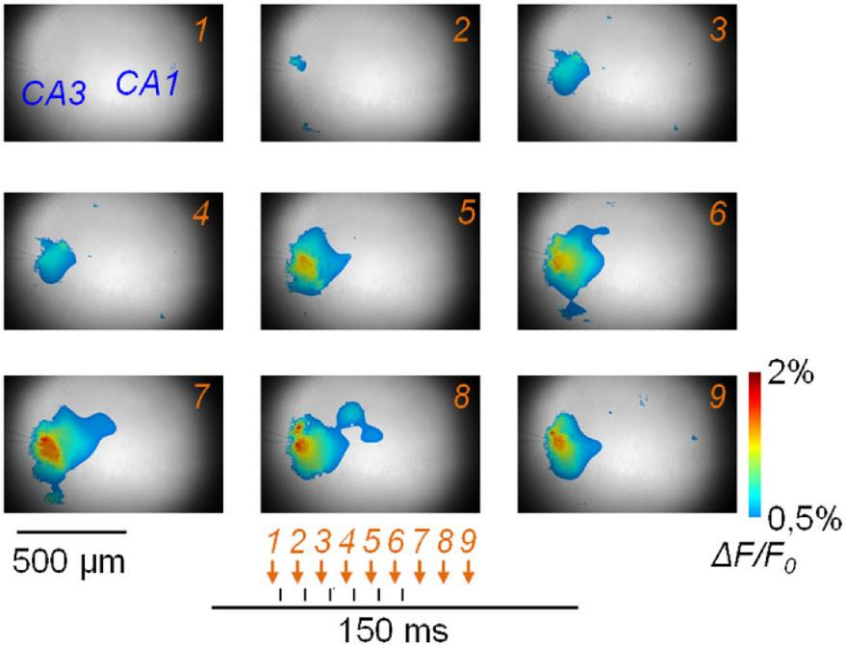
A decision as important as the choice of indicator is the optical system with which fluorescence will be collected. Each platform strikes a different balance among spatial resolution, temporal resolution, imaging depth, field of view and experimental flexibility; none is superior to the others in every respect. All of these methods share one cost. Light gradually destroys the indicator molecules (photobleaching), so the signal fades during long recordings, and light also damages the tissue itself (phototoxicity), so cells become less healthy the longer they are illuminated. How much of the sample is exposed to light, and for how long, is therefore an important difference between platforms.

3.1. Wide-Field Imaging

In the simplest configuration, the sample is illuminated in its entirety by an LED, an arc lamp or a laser, and the fluorescence is focused onto a camera. Because no scanning is involved, wide-field imaging (camera-based epifluorescence) is the fastest platform available: the frame rate is limited only by the readout speed of the camera and the number of photons available. Modern scientific CMOS (sCMOS) cameras deliver hundreds of frames per second at full

frame and thousands in cropped regions. The price is the absence of optical sectioning: out-of-focus fluorescence contaminates the image, so the method performs best in thin preparations (cell cultures, the surface layers of acute slices) or in sparsely labeled tissue. Even so, for the simultaneous recording of evoked synaptic responses or action-potential-driven calcium transients from many sites in acute brain slices, wide-field camera imaging is the approach that reaches kilohertz rates over large fields (Abbas, İpek, Moreau, & Canepari, 2025).

Figure 1. Fast population calcium signal propagating from CA3 to CA1 in a hippocampal slice loaded with Fluo-4 AM, recorded with wide-field camera imaging.



Nine consecutive frames (10-ms interval) following electrical stimulation are shown; ΔF/F₀ (change in fluorescence relative to the pre-stimulus baseline) is color-coded over the transmitted-light image and values below 0.5% are not displayed. Arrows below indicate the timing of each frame relative to the stimulus train. Note the small signal amplitude (0.5–2%) and the millimeter-scale field of view. Reproduced from İpek et al. (2025), European Journal of Neuroscience, under a CC BY 4.0 license.

3.2. Confocal and Two-Photon Microscopy

Confocal microscopy achieves optical sectioning by rejecting out-of-focus light with a pinhole; however, the entire depth of the sample is still excited, so photobleaching and phototoxicity accumulate throughout the illuminated column, point-by-point scanning makes it slow, and excitation with visible light limits its depth in scattering tissue. Two-photon microscopy (Denk, Strickler, & Webb, 1990) solves these problems with a different physical principle: the

indicator is excited by the simultaneous absorption of two near-infrared photons, each carrying half the energy, and therefore twice the wavelength, of the single visible photon that would normally be needed. Because two photons are only likely to arrive together where light is most concentrated, excitation is confined to the focal volume; near-infrared light also scatters less in tissue than visible light, and photodamage does not occur outside the focus. As a result, two-photon microscopy makes cellular-resolution imaging possible at depths exceeding 500 μm in the living mouse cortex and has become the standard for in vivo calcium imaging (Helmchen & Denk, 2005; Stosiek et al., 2003). Three-photon excitation takes the same logic one step further, using three photons of even longer wavelength that scatter less still, allowing non-cortical structures such as the hippocampus to be reached without removing the overlying tissue (Ouzounov et al., 2017).

The principal limitation of two-photon microscopy is speed. With standard galvanometric scanners the full-frame (512 × 512 pixel) rate is only a few hertz; resonant scanners raise this to about 30 Hz, and reduced fields of view or line scans reach a few hundred hertz. Random-access scanning with acousto-optic deflectors, which jumps between pre-selected cell bodies, has made it possible to sample tens of neurons at hundreds of hertz and to estimate action potential timing with near-millisecond precision (Grewe, Langer, Kasper, Kampa, & Helmchen, 2010). As discussed in Section 4, these approaches have now been pushed into the kilohertz range.

3.3. Miniaturized Microscopes

Head-mounted single-photon miniscopes weighing a few grams (Ghosh et al., 2011) have carried calcium imaging into freely moving animals. Deep structures (hippocampus, striatum, amygdala) can be reached through a Gradient Refractive Index (GRIN) lens, and the same neuronal population can be followed for weeks, a capability that has transformed studies of learning, memory and social behavior. The open-source UCLA Miniscope project reduced cost and broadened adoption (Aharoni & Hoogland, 2019). The price of single-photon excitation is the same as in wide-field imaging: there is no optical sectioning, so fluorescence from the neuropil (the dense mesh of axons and dendrites surrounding the cell bodies, which is also labeled and also active) mixes with the signal of each cell and no z-axis information is obtained. Direct comparisons in which the same neurons were imaged with both a miniscope and a bench-top two-photon system found that response amplitudes and signal-to-noise ratios were highly correlated between the two methods, although miniscope data were more affected by neuropil contamination (Glas, Hübener, Bonhoeffer, & Goltstein, 2019). These limitations motivated the development of two-photon miniscopes based on MEMS scanners and hollow-core photonic crystal fibers; current designs weigh under 3 g, offer

fields of view of up to 500 × 500 µm with a 240 µm z-scanning range, and record more than 1000 neurons at a time in freely moving mice (Zong et al., 2022).

3.4. Light-Sheet and Light-Field Microscopy

In transparent organisms such as the larval zebrafish, light-sheet microscopy illuminates the sample from the side with a thin plane of light and images it with a camera at right angles, capturing the entire brain of the larval zebrafish (less than a millimeter across) at cellular resolution roughly once per second (Ahrens, Orger, Robson, Li, & Keller, 2013). Light-field microscopy encodes volumetric information into a single camera frame through a microlens array, yielding three-dimensional images without scanning; spatial resolution is sacrificed, but the volume rate equals the camera frame rate. These approaches remain limited in the scattering tissue of the mammalian brain, but scanning and confocal variants have begun to be applied in mouse cortex (Nguyen et al., 2025). Table 2 summarizes the main features of the platforms discussed.

Table 2. Comparison of the principal imaging platforms

Platform	Optical sectioning	Frame rate (Hz)[a]	Imaging depth (µm)[b]	Preparation	Limitation
Wide-field	None	100-1000	50-100	Cultures, Acute slices	Out-of-focus background
Confocal	Pinhole	1-30	50-150	Cultures, Acute slices	Photobleaching, Slow scanning
Two-photon	Intrinsic	1-30	500-1000	Head-fixed in vivo, Acute slices	Cost, Scan-limited frame rate
Single-photon miniscope	None	20-60	100-200	Freely moving in vivo	Neuropil contamination
Two-photon miniscope	Intrinsic	10-40	150-200	Freely moving in vivo	Head-mounted mass, Fiber tether
Light-sheet	Illumination plane	1-10	200-300	Larval zebrafish	Requires transparent tissue
Light-field	Computational	10-100	100-200	Larval zebrafish, Mouse cortex	Reduced spatial resolution

[a] *At full field of view; cropping or line scanning raises wide-field and two-photon systems into the kilohertz range (Section 4). For light-sheet and light-field microscopy the value is the volume rate.*

[b] *Below the objective or, for miniscopes, below the face of the implanted Gradient Refractive Index (GRIN) lens through which deep structures are reached.*

Compiled by the author from Ahrens et al. (2013), Glas et al. (2019), Zong et al. (2022) and Nguyen et al. (2025). Values indicate typical ranges and vary with the specific system.

4. THE TRADE-OFF AMONG SPEED, RESOLUTION AND SENSITIVITY

4.1. The Photon Budget

The constraint common to all optical imaging methods is that photons are countable and finite. At a given illumination intensity, the number of photons collected from a cell per unit time is fixed; when the frame rate is increased, fewer photons fall in each frame and photon shot noise grows as the square root of the count. From this principle follow the theoretical limits on the optical detection and timing of action potentials: signal amplitude (ΔF/F), indicator kinetics and the number of collected photons together determine the fastest event that can be detected with a given reliability (Wilt, Fitzgerald, & Schnitzer, 2013). Increasing illumination yields more photons, but photobleaching and photodamage increase in proportion, so a balance must be struck between long recordings and high speed.

In camera-based systems, optical demagnification and pixel binning concentrate more photons into each effective pixel, while cropping the field of view shortens the camera's readout time; both raise the achievable frame rate. In scanning systems, reducing the number of scanned points (random access, line scanning) achieves the same goal. In both cases, the speed gained is paid for with spatial information.

4.2. Camera Technology and Bit Depth

Scientific cameras have evolved from CCD to electron-multiplying CCD (EMCCD) and then to sCMOS. EMCCDs offer superior sensitivity under very low light, but their readout speeds and pixel counts are limited; sCMOS sensors combine high quantum efficiency, a wide field of view and high frame rates and have become the standard detector for fast calcium imaging. On the horizon are photon-number-resolving CMOS sensors that count individual photons and largely eliminate readout noise (Nguyen et al., 2025).

A less frequently discussed parameter is bit depth. Most cameras operate with a 12 or 16-bit dynamic range, but at high frame rates the data transfer bandwidth becomes limiting, and a choice must be made between frame rate and frame size. Acquiring at 8-bit depth and binning pixels offline relaxes this constraint: in brain slices this approach resolved population calcium transients at 2 kHz from large frames, dendritic calcium signals associated with a back-propagating action potential at 10 kHz, and an action potential in the axon initial segment at 40 kHz with a voltage-sensitive dye, all on a standard epifluorescence microscope equipped with a large-format sCMOS camera (Abbas et al., 2025).

4.3. Kilohertz Imaging

Given that neuronal firing rates range from 0.1 Hz in cortical pyramidal cells to 500 Hz in fast-spiking interneurons, resolving action potential trains reliably requires sampling in the kilohertz range. Three strategies for reaching this goal can be distinguished. The first is kilohertz two-photon microscopy: techniques such as grid-foci scanning, acousto-optic random access, scanned line angular projection tomography (SLAP) and free-space angular-chirp-enhanced delay (FACED) have achieved planar recording rates of 1–10 kHz in awake mice. The second comprises hybrid approaches that combine the volumetric speed of light-field microscopy with event-based cameras. The third is event-based imaging itself: rather than producing images at a fixed frame rate, these cameras asynchronously report only those pixels whose brightness changes, reducing data redundancy and raising the effective temporal resolution into the kilohertz range (Nguyen et al., 2025).

The common outcome of these developments is that optical recordings are approaching electrophysiology in the temporal domain. A caveat is needed here, however: the intrinsic kinetics of the calcium indicator impose a lower bound regardless of sampling rate. Sub-millisecond sampling becomes meaningful only with fast indicators such as jGCaMP8, or with direct voltage sensors. That is the subject of the next section.

5. FROM CALCIUM TO VOLTAGE

5.1. The Indirect Nature of the Calcium Signal

However fast calcium imaging becomes, what it measures is not the membrane potential but one of its consequences. This indirectness has concrete implications. Subthreshold synaptic potentials, hyperpolarizations that do not trigger calcium entry and inhibitory inputs are either invisible in the calcium signal or only weakly reflected. During high-frequency firing, the calcium transients of successive action potentials summate and the indicator approaches saturation, so that individual events can no longer be distinguished. Moreover, the amplitude of a calcium transient depends on cell type, compartment and the momentary state of the cell; the same number of action potentials can produce different $\Delta F/F$ in different cells. For these reasons, voltage imaging, which converts membrane potential directly into fluorescence, has long been regarded as the missing half of calcium imaging (Knöpfel & Song, 2019).

5.2. Voltage Indicators

Voltage-sensitive dyes (VSDs) have been in use since the 1970s; these molecules insert into the membrane and their fluorescence changes with the electric field. Although they offer sub-millisecond kinetics, they lack cell-type selectivity, stain all membranes including glia and are strongly phototoxic. Genetically encoded voltage indicators (GEVIs) were developed to overcome

these limitations and fall into three main families (Nguyen et al., 2025). The first family couples a voltage-sensing domain derived from a phosphatase to a fluorescent protein; ArcLight and the ASAP series are its best-known members. ASAP3 made kilohertz two-photon voltage imaging possible in awake mice (Villette et al., 2019), and the ASAP4 and ASAP6 variants together with JEDI-2P have pushed sensitivity and photostability further. The second family exploits either the weak but voltage-dependent fluorescence of microbial rhodopsins or electrochromic FRET between a rhodopsin and a fluorescent protein; Archon and Ace-mNeon derivatives belong to this group. The third family consists of chemigenetic sensors, in which a synthetic, bright and photostable dye is attached to a genetically targeted protein scaffold. Voltron and its derivatives are the most successful examples of this approach; in mouse cortex, Voltron enabled single-trial recording of spikes and subthreshold signals from dozens of neurons over 15 minutes of continuous imaging in awake mice (Abdelfattah et al., 2019).

5.3. Recent Developments and Limits

A notable trend of recent years is red-shifted GEVIs. Inserting a red fluorescent protein into the extracellular loop of a rhodopsin scaffold produced the Cepheid1b and Cepheid1s sensors, with which burst firing and subthreshold depolarizations were recorded in acute brain slices at low laser power (Han et al., 2023). The particular value of red GEVIs lies in their spectral separation from a green calcium indicator: voltage and calcium can be imaged simultaneously in the same cell. The same study illustrated this by demonstrating cell-to-cell heterogeneity in voltage–calcium coupling in pancreatic islets. Similarly, multiplexing two GEVIs of opposite response polarity has allowed the concurrent voltage dynamics of distinct interneuron populations to be recorded in awake mice (Kannan et al., 2022).

Despite these advances, voltage imaging has not yet reached the maturity of calcium imaging. The voltage signal originates only from the plasma membrane; far fewer molecules contribute than in the case of the calcium signal arising from the cytosolic volume of the soma, and $\Delta F/F$ values per action potential are typically in the range of 10–50%. The requirement for kilohertz sampling narrows the photon budget, so the signal-to-noise ratio is low, and photobleaching limits recording duration to minutes. Under two-photon excitation the performance of many GEVIs falls markedly, and membrane-targeting efficiency varies from sensor to sensor. In short, voltage imaging is a complement to, not a replacement for, calcium imaging: calcium is preferred for the long-term population dynamics of many cells in deep tissue, whereas voltage is preferred for the short-term analysis of subthreshold events and precise spike timing in selected cells.

6. DATA ANALYSIS

6.1. Pre-processing and Source Extraction

A raw calcium imaging recording consists of thousands of frames, each of which may be tens of megabytes; reaching meaningful information requires a multi-stage processing pipeline. The first step is motion correction: in recordings made in vivo, respiration, heartbeat and the animal's movements cause shifts in the image that are corrected with rigid or piecewise-rigid registration algorithms. The second step is the identification of cells (regions of interest) and the separation of each cell's time series from the background. Hand-drawn regions of interest are still used for small data sets, but automated methods are standard for large-scale recordings: principal and independent component analysis (Mukamel, Nimmerjahn, & Schnitzer, 2009) and constrained non-negative matrix factorization (CNMF; Pnevmatikakis et al., 2016) are the two foundational approaches. The Suite2p (Pachitariu et al., 2016; Stringer & Pachitariu, 2024) and CaImAn (Giovannucci et al., 2019) packages, which turn these methods into end-to-end pipelines, have become de facto standards in the field thanks to their open-source nature and their capacity to process tens of thousands of neurons. Variants such as CNMF-E, with an extended background model, have been developed for single-photon miniscope data.

The third step is the normalization of fluorescence. The most common measure, $\Delta F/F$, is the deviation of fluorescence at each time point from baseline, divided by the baseline; the baseline is usually estimated as a low percentile within a sliding window. This simple ratio allows different cells and recordings to be compared, but neuropil contamination, baseline drift due to photobleaching and the baseline estimate itself can all bias $\Delta F/F$ values systematically. High-speed recordings additionally require temporal filtering and averaging across trials; here, choosing the filter bandwidth according to the indicator's kinetics sets the balance between smoothing the signal and suppressing noise.

6.2. Spike Inference

$\Delta F/F$ traces are not neuronal activity itself but a version of it convolved with the kinetics of the calcium indicator. Recovering action potential times or firing rates from such traces is called spike inference and is among the most demanding computational problems in the field. Model-based approaches describe the calcium transient with an exponential kernel and apply deconvolution; the OASIS algorithm used in Suite2p and CaImAn, and the more elaborate Bayesian model MLSpike, are representatives of this class. Their common drawback is that parameters such as indicator kinetics and noise level must be tuned separately for each data set.

Supervised deep learning has brought a different solution to this problem. CASCADE, an algorithm trained on a large ground-truth database of

simultaneous electrophysiological and calcium recordings from the same neurons, outperformed model-based methods without any parameter tuning by the user (Rupprecht et al., 2021). The algorithm automatically selects a model appropriate to the noise level and sampling rate of each neuron. The distinct linearity and kinetics of the jGCaMP8 sensors have required such tools to be re-evaluated; a recent analysis established the need for models retrained on GCaMP8 data and the decisive role of indicator linearity in inference accuracy (Rupprecht et al., 2026). The same study shows that the fast rise time of jGCaMP8 also enables real-time spike detection in closed-loop experiments. This is a concrete example of the need for indicator development and analysis methods to evolve together.

6.3. Denoising and Deep Learning

Another application of deep learning is the denoising of images acquired under low-photon conditions. Self-supervised methods such as DeepInterpolation (Lecoq et al., 2021) and DeepCAD (Li et al., 2021) exploit the principle that consecutive frames contain independent noise but correlated signal and can improve the signal-to-noise ratio by an order of magnitude (10 to 15-fold in single-pixel SNR) without requiring labeled data. These approaches are being adopted rapidly, particularly in voltage imaging, as a way of compensating for the restricted photon budget in software. It should be borne in mind, however, that self-supervised denoising carries the risk of generating structures that do not actually exist, based on learned priors, and that results should always be validated with an independent method.

6.4. Blind Spots

One of the entrenched assumptions of calcium imaging analysis is that neuronal activity appears in fluorescence only as an increase; CNMF-based source extraction and most spike inference algorithms therefore decompose the signal into non-negative components. It has been shown systematically that this assumption renders inhibited neurons invisible: in a spontaneously active cell, inhibition produces a fluorescence decrease below baseline, and the non-negativity constraint either erases this decrease or interprets the return of fluorescence to baseline as a spurious spike (Vanwalleghem, Constantin, & Scott, 2020). The authors recommend that, before any pipeline is applied, the presence of meaningful negative deviations be examined with unconstrained exploratory methods such as k-means clustering or principal component analysis. This example is a reminder that even the most widely used tools must be chosen critically in light of the experimental question.

7. CONCLUSION AND FUTURE DIRECTIONS

Within two decades, the optical imaging of neuronal activity has grown from the specialty of a few laboratories into a core infrastructure of neuroscience. The

driving force behind this transformation has not been a single technology but three mutually reinforcing lines of development: indicators that have brought speed and sensitivity to the millisecond scale, platforms that range from free behavior to kilohertz recording, and analysis tools that make large data sets interpretable. An investigator today faces a broad menu of options, and the right choice depends on the scale of the experimental question. If hundreds of cells must be followed for weeks, GECIs and miniscopes are indicated; if the activity of defined cell types in deep tissue is at issue, two-photon microscopy; if sub-millisecond analysis of synaptic or dendritic events in acute slices is the goal, high-speed wide-field imaging with synthetic dyes; and if subthreshold dynamics and precise spike timing are required, voltage imaging.

Several trends for the near future are already apparent. The first is multicolor and multimodal imaging: the simultaneous use of calcium, voltage, neurotransmitter and neuromodulator sensors in the same preparation will reveal causal relationships that no single measurement can. The second is all-optical electrophysiology, in which imaging and optogenetic manipulation are combined along the same optical path; spectrally separated red sensors are opening the way to this combination. The third is the co-design of hardware and software: event-based cameras, high-speed low-bit-depth acquisition and self-supervised denoising are complementary strategies that push against the photon-budget limit from different directions. Finally, the joint development of indicator engineering and analysis methods, as seen in spike inference models retrained for jGCaMP8, is a sign that the field is maturing.

Optical imaging has not replaced electrophysiology and will not do so; the two approaches validate and complement each other. Yet the ability to see neuronal activity together with cell identity, spatial arrangement and change over time has opened a window onto the question of how the nervous system computes that was previously inaccessible. How far this window widens will depend, in the coming years, on how closely advances in indicators, optics and computation become interlocked.

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CHAPTER 6

Perianal Fistula: Integrating Anatomy, Pathophysiology, and Precision Surgical Strategies; An Evidence-Based Approach to Diagnosis, Sphincter Preservation, and Functional Outcomes

***Ali Bekraki*[1]**

***Ali Levent Işık*[2]**

[1] MD., Assistant professor of surgery University of Health Sciences, Hamidiye Medical School, Department of General Surgery, Üsküdar – İstanbul/Turkiye.
0000-0002-9396-6124

[2] MD., Specialist of General Surgery, Çam And Sakura City Hospital, Başakşehir-İstanbul, 0000-0002-5368-2207

1. Introduction

Perianal fistula is a complex anorectal disorder characterized by an abnormal connection between the anal canal and perianal skin, representing the chronic phase of a cryptoglandular abscess. Approximately 30-40% of patients develop a persistent fistula following the incision and drainage of an acute abscess, leading to chronic discharge, pain, and impaired quality of life [1]. The condition maintains a stable annual incidence of 1–2 cases per 10,000 individuals, predominantly affecting males in the third to fifth decades of life [2, 3]. While most cases are cryptoglandular, a significant subset is associated with systemic pathologies, most notably Crohn's disease [4].

Historically, the management of perianal fistulae has oscillated between the invasive maneuvers described by Hippocrates and the conservative pharmacological "drying" advocated by 13th-century Arab physicians [5, 6]. Since the landmark surgical success of Charles-François Félix on Louis XIV in 1686, the field has transitioned into a precision-based discipline [7].

The central challenge of modern proctology remains the delicate balance between definitive tract eradication and the absolute preservation of fecal continence. While conventional fistulotomy remains the gold standard for simple cases, its application in complex tracts carries a prohibitive risk of sphincter injury. Consequently, the current therapeutic landscape has shifted toward sphincter-preserving and minimally invasive techniques, necessitating an evidence-based framework to optimize functional outcomes and minimize recurrence.

2. Surgical Anatomy of the Anal Canal and Pelvic Floor

A detailed understanding of the anal canal and pelvic floor is fundamental for safe and effective management of perianal fistulas.

2.1 Internal Anal Sphincter

The internal anal sphincter (IAS) is a continuation of the rectal circular smooth muscle, extending 2–4 cm from the dentate line to the anal verge [8]. It functions involuntarily under autonomic control and contributes the majority of resting anal tone.

Histological and imaging studies suggest sex-related variation in anal sphincter morphology, with relatively greater anterior sphincter prominence in females and more pronounced posterior muscular bulk in males [9]. Injury to the IAS can reduce resting tone, increasing the risk of passive incontinence, particularly in inter-sphincteric and low trans-sphincteric fistulas.

2.2 External Anal Sphincter

The external anal sphincter (EAS) is striated, voluntary muscle that maintains active continence during exertion or conscious contraction. It is divided into three segments:

- Subcutaneous layer – superficial, encircling the anal verge, easily palpable.
- Superficial layer – arises from the perineal body and blends anteriorly with the puborectalis, contributing to sphincter closure.
- Deep layer – surrounds the upper anal canal and interdigitates with the puborectalis and levator ani, coordinating continence with rectal evacuation.

Preservation of the EAS is essential during fistula surgery. Damage can result in stress incontinence, especially in high or trans-sphincteric tracts, making sphincter-sparing techniques crucial.

2.3 Puborectalis Muscle

The puborectalis forms the medial portion of the levator ani and functions as a muscular sling around the anorectal junction. It originates from the posterior aspect of the pubic symphysis and loops posteriorly around the anorectal junction, inserting to the posterior surface of the pubic bones on both sides. This arrangement creates a functional valve that maintains the anorectal angle (~80–90° at rest) and regulates defecation [10].

The puborectalis works synergistically with the IAS and EAS to maintain continence. Recognition of its anatomy is critical when addressing supra-sphincteric or high trans-sphincteric fistulas, as injury can disrupt the anorectal angle and compromise function.

2.4 Pelvic Floor and Surgical Landmarks

The levator ani complex, consisting of the puborectalis, pubococcygeus, and iliococcygeus muscles, provides structural support to the rectum and anal canal. Key landmarks for surgical planning include:

Pelvic brim – reference point for supralevator abscesses or high fistula tracts.

Anorectal angle – maintained by the puborectalis; integrity is essential for continence.

2.5 Perianal and Perirectal Spaces:

These potential spaces define the routes through which infection spreads from the anal glands and determine the anatomical classification of fistulous tracts.

2.5.1 Intersphincteric Space

The intersphincteric space, situated between the internal and external anal sphincters, is the most common site of origin for cryptoglandular infection. It extends longitudinally along the anal canal and communicates superiorly with the supralevator space. Infection within this plane may track distally to the perianal skin, resulting in an intersphincteric fistula, the simplest and most frequent form. Lateral extension through the external sphincter gives rise to a transsphincteric fistula, while superior spread into deeper compartments leads to more complex disease patterns. Accurate identification of the internal opening within this space is fundamental to surgical management, as procedures such as fistulotomy and ligation of the intersphincteric fistula tract (LIFT) rely on precise anatomical localization.

2.5.2 Ischioanal (Ischiorectal) Fossa

The ischioanal fossa is a fat-filled space located lateral to the anal canal and inferior to the levator ani. Its loose structure permits rapid lateral spread of infection. Involvement of this space is characteristic of trans-sphincteric fistulas, where infection penetrates the external sphincter and extends into the fossa. Posterior communication via the deep postanal space may result in horseshoe fistulas, often involving both sides.

2.5.3 Supralevator Space

The supralevator space lies above the levator ani and represents a deep pelvic compartment. Infection may reach this space through upward extension from the intersphincteric plane or, less commonly, from pelvic sources.

Fistulas involving this space are typically suprasphincteric or extrasphincteric and are often complex. Clinical detection is challenging, making pelvic MRI essential for accurate diagnosis and mapping. Surgical management requires careful planning to avoid creating additional tracts or compromising sphincter function.

2.5.4 Deep Postanal Space

The deep postanal space, located posterior to the anal canal, connects the two ischioanal fossae. It plays a central role in the development of posterior complex fistulas. Infection within this space can lead to horseshoe abscesses, which may extend bilaterally. Recognition of this space is crucial, as incomplete drainage frequently results in recurrence. Surgical treatment often requires wide drainage and, in some cases, staged procedures.

2.5.5 Perianal Space

The perianal space is the most superficial compartment, located immediately beneath the perianal skin. It is typically involved in simple abscesses and low

fistulas. Management is usually straightforward, involving incision and drainage or fistulotomy, with minimal risk to sphincter integrity when appropriately performed.

3. Pathophysiology of Perianal Fistula Formation

Perianal fistula results from a combination of infection, persistent inflammation, and impaired healing. The cryptoglandular hypothesis remains the principal mechanism underlying most cases.

3.1 Cryptoglandular Origin

According to the classic model described by Parks, infection begins in the anal glands at the dentate line, which traverse the internal sphincter into the intersphincteric space [11,12]. Obstruction of these glands leads to abscess formation, with subsequent extension along anatomical planes, resulting in fistula tract development toward the perianal skin.

3.2 Abscess–Fistula Continuum

The transition from acute sepsis to a chronic fistula is driven by the pressure gradient between the anal canal and the perianal skin. Failure to obliterate the offending anal crypt during initial drainage allows the inflammatory track to mature into a stable, collagenous tunnel. Consequently, identifying and managing this internal opening—the "engine" of the fistula—is the critical determinant in preventing recurrence and achieving definitive surgical success.

3.3 Microbial Dysbiosis and the Inflammatory Cascade

The pathogenesis of perianal fistula involves a synergistic interaction between microbial activity and dysregulated host immunity. While E. coli and Bacteroides species drive acute abscess formation, chronic fistulization is increasingly linked to dysbiotic biofilm formation within the anal glands [11]. This biofilm acts as a reservoir, sustaining a chronic inflammatory milieu characterized by the upregulation of TNF-α, IL-1β, and IL-8 even in the absence of overt clinical infection [13-15]. These cytokines facilitate continuous tissue degradation and impair mucosal regeneration, ultimately preventing tract healing and leading to clinical persistence.

3.4 Tissue Remodeling and Tract Persistence

Fistula tracts are often lined by cells exhibiting mesenchymal features rather than normal epithelium, likely as a result of epithelial–mesenchymal transition (EMT). Through this process, epithelial cells acquire increased migratory capacity and invasive behavior, contributing to the persistence of the fistulous tract and preventing normal closure.

Increased activity of matrix metalloproteinases (MMPs) further promotes extracellular matrix degradation and tract extension, while cytokines such as

TGF-β and IL-13 regulate these remodeling processes [16]. Recognition of EMT-driven tissue remodeling has important therapeutic implications, as emerging experimental therapies aim to modulate these molecular pathways in order to restore epithelial healing and promote fistula closure.

3.5 Secondary Causes of Perianal Fistula

Although most perianal fistulas are cryptoglandular in origin, a subset arises secondary to underlying systemic, infectious, or local conditions. These cases are often associated with more complex, recurrent, or atypical disease and require targeted management of the underlying cause in addition to surgical treatment.

Common secondary causes include:

- Crohn's disease – characterized by transmural inflammation and epithelial barrier disruption, leading to deep, recurrent, and often multiple fistulas.
- Tuberculosis – causes granulomatous inflammation and should be considered in endemic regions or atypical presentations.
- Malignancy – may present with fistula-like tracts due to tumor infiltration or necrosis, particularly in anorectal cancers.
- Radiation injury – results in tissue ischemia, fibrosis, and impaired healing, predisposing to fistula formation.

Additional less common causes include:

- Other infections – such as actinomycosis, HIV-associated infections, and sexually transmitted infections (e.g., lymphogranuloma venereum, syphilis), which may present with atypical, multiple, or non-healing fistulas.
- Inflammatory and systemic conditions – including hidradenitis suppurativa, which can involve the perianal region and produce sinus tracts that mimic or coexist with fistulas, and rarely sarcoidosis.
- Post-surgical or traumatic causes – including iatrogenic injury following anorectal procedures, obstetric trauma, or penetrating injury.

4. Classification of Perianal Fistulas

Accurate classification of perianal fistulas is essential for diagnosis, surgical planning, and outcome prediction. Among several proposed systems, the classification described by Parks et al. remains the most widely used, categorizing fistulas according to their relationship with the anal sphincter complex.

4.1 Parks Classification

The Parks classification describes four principal types of fistulas based on the course of the fistulous tract relative to the internal and external anal sphincters [17].

Intersphincteric Fistula

Intersphincteric fistulas are the most common subtype, accounting for approximately 60–70% of cases [18]. The tract originates at the internal opening in the anal canal and courses within the intersphincteric plane before opening onto the perianal skin. As the external sphincter is not involved, these fistulas are usually amenable to simple surgical treatment such as fistulotomy.

Transsphincteric Fistula

Transsphincteric fistulas account for approximately 20–30% of cases [18]. The tract traverses both the internal and external anal sphincters and extends through the ischioanal fossa to the perianal skin. Because a portion of the external sphincter is involved, surgical management must aim to eradicate the tract while preserving continence.

Suprasphincteric Fistula

Suprasphincteric fistulas are uncommon. The tract originates in the intersphincteric space, ascends above the puborectalis muscle, and then descends through the levator ani to the perianal skin. Owing to their high course, these fistulas are generally classified as complex and require careful surgical planning.

Extrasphincteric Fistula

Extrasphincteric fistulas are rare and do not arise from the anal glands. The tract extends from the rectum or sigmoid colon to the perianal skin without traversing the sphincter complex. They are typically associated with secondary causes such as trauma, inflammatory bowel disease, malignancy, or pelvic infection [11, 17].

4.2 Simple versus Complex Fistulas

Perianal fistulas are broadly categorized as simple or complex, a distinction that informs surgical strategy and helps predict functional outcomes.

Simple fistulas are generally low-lying, including intersphincteric or low transsphincteric tracts that involve minimal sphincter muscle. They lack secondary branches, multiple openings, or associated systemic disease. Due to their limited anatomical involvement, these fistulas are usually amenable to fistulotomy with a low risk of fecal incontinence.

Complex fistulas involve more extensive sphincter or pelvic floor structures and present additional surgical challenges. Features that define complexity include:

High transsphincteric, suprasphincteric, or extrasphincteric tracts

Multiple internal or external openings

Anterior location in females, where sphincter preservation is critical

Recurrent disease

Underlying systemic or local pathology, such as Crohn's disease, tuberculosis, malignancy, or prior radiation

Secondary branches or extensions into the ischioanal or supralevator spaces

These anatomical and clinical factors are associated with a higher risk of recurrence and sphincter injury, often necessitating staged, sphincter-preserving, or tailored surgical approaches. Preoperative recognition of these features, aided by detailed imaging, is essential to optimize treatment planning, minimize functional compromise, and achieve durable outcomes.

4.3 Additional Descriptive Systems

Beyond traditional clinical classification, the St. James's University Hospital system leverages MRI to delineate complex fistula anatomy [19]. By detailing the relationship between tracts, secondary extensions, and associated abscesses relative to the sphincter complex, this imaging-based approach facilitates precise preoperative mapping. Such detail is critical for optimizing surgical outcomes and minimizing inadvertent injury to the continence mechanism.

Table 1: Comparison of the Parks and St. James's MRI Classifications of Perianal Fistulas.

Aspect	Parks Classification	St. James's MRI Classification
Basis	Surgical anatomy	MRI imaging findings
Focus	Relationship to sphincter complex	Detailed tract anatomy + extensions
Main Types	Intersphincteric, Transsphincteric, Suprasphincteric, Extrasphincteric	Grades 1–5 (increasing complexity)
Detection of secondary tracts	Limited	Clearly identifies secondary extensions
Abscess identification	Clinical/surgical	Accurately detected on MRI
Use in practice	Intraoperative and conceptual classification	Preoperative planning and mapping
Strengths	Simple, widely used, surgically intuitive	Precise, detects occult disease
Limitations	May miss complex extensions	Less intuitive without imaging

5. Diagnosis: A Multi-Modal Precision Approach

5.1 Clinical Evaluation: The Functional and Anatomical Baseline

Clinical evaluation remains the primary tool for assessing functional baseline and symptomatic burden. The external examination focuses on identifying the primary opening, peri-fistular induration, and evidence of prior surgical scarring. Palpation of an "indurated cord" extending toward the anal canal provides immediate trajectory insight, traditionally guided by Goodsall's Rule: external openings posterior to a transverse anal line generally follow a curved path to the posterior midline, whereas anterior openings typically follow a radial, direct path.

Digital Rectal Examination is mandatory for assessing sphincter tone and preoperative risk. It aims to localize the internal opening at the dentate line and detect high-tract fluctuance indicative of supralevator extension. However, clinical evaluation and Goodsall's Rule frequently fail to identify complex "horseshoe" extensions or deep-seated abscesses [11, 20]. These diagnostic limitations mandate the integration of 3D-EAUS and MRI to ensure a precision-based strategy that prioritizes both tract eradication and the preservation of fecal continence [11, 21].

5.2 Imaging Modalities

5.2.1 Endoanal Ultrasound (EAUS) and 3D Imaging

Endoanal ultrasound (EAUS) provides high-resolution, real-time visualization of the anal sphincter complex, serving as a vital diagnostic tool for defining the anatomy of simple and low-lying fistulas. The emergence of 3D-EAUS has significantly enhanced diagnostic precision, as it allows for multiplanar reconstruction and a more detailed volumetric assessment of the intersphincteric and transsphincteric planes [22]. In many clinical scenarios, 3D-EAUS is considered superior to MRI for the exact "clock-face" localization of the internal opening, particularly in simple or low transsphincteric tracts where mucosal detail is paramount [22, 23].

The clinical application of EAUS is further optimized through the use of hydrogen peroxide (H_2O_2) contrast enhancement. When injected into the external opening, H_2O_2 produces a hyperechoic effect that clearly delineates the primary tract and identifies subtle secondary branches that might otherwise be missed. Despite these strengths, EAUS remains highly operator-dependent and possesses inherent physical limitations, specifically a limited depth of penetration. Consequently, its diagnostic accuracy diminishes when evaluating complex cephalad extensions, such as high suprasphincteric or supralevator tracts, which typically necessitate the broader field of view provided by pelvic MRI.

5.2.2 Magnetic Resonance Imaging (MRI)

Pelvic MRI is the gold standard for high-resolution mapping of complex perianal disease [24, 25]. Its superior soft-tissue contrast provides a definitive anatomical roadmap of primary tracts, associated abscesses, and intricate secondary extensions. Unlike endoanal ultrasound, MRI offers a broader field of view, making it essential for detecting occult supralevator involvement and multi-branched "horseshoe" tracts that may elude clinical detection.

MRI facilitates a standardized diagnostic approach by integrating the St. James's University Hospital classification. This system complements the Parks classification by providing a radiologically precise assessment of the tract's relationship to the pelvic floor, which is critical for preoperative planning. Furthermore, MRI is invaluable for evaluating associated pathologies, such as transmural inflammation in Crohn's disease or fibrotic changes following radiation. By accurately delineating sphincter involvement, MRI serves as the keystone of precision surgical decision-making, optimizing sphincter-preserving strategies to minimize postoperative fecal incontinence.

5.3 Endoscopic Evaluation

Endoscopic assessment, including flexible sigmoidoscopy or total colonoscopy, serves as a vital diagnostic adjunct in the management of recurrent or atypical fistulas. Its primary role is to exclude secondary etiologies such as Crohn's disease, intestinal tuberculosis, or anorectal malignancy, which fundamentally alter the management paradigm. Furthermore, endoscopy is essential for detecting localized proctitis or occult internal mucosal lesions that may not be apparent during physical examination. Identifying these underlying conditions ensures the surgical approach is tailored to the tissue environment, thereby reducing the risk of postoperative healing failure.

5.4 Diagnostic Integration and Classification Synergy

The definitive classification of a perianal fistula requires a strategic integration of clinical, endoscopic, and radiological findings. While simple, low-lying intersphincteric tracts may be adequately mapped via 3D-EAUS and physical examination, complex disease—including high transsphincteric, recurrent, or suprasphincteric tracks—mandates high-resolution pelvic MRI. This multi-modal assessment is the critical determinant in localizing the primary internal opening, delineating secondary "horseshoe" branches, and identifying occult supralevator extensions. By accurately mapping this anatomy, the surgeon can transition from generalized management to a precision-based strategy, specifically tailoring the choice between obliterative and sphincter-preserving interventions to minimize recurrence and optimize functional continence.

6. Principles of Surgical Management

The management of perianal fistula necessitates a precision-based equilibrium between definitive tract eradication and the absolute preservation of fecal continence. The primary objective is the total obliteration of the primary tunnel and secondary branches while minimizing iatrogenic trauma to the sphincter complex [26]. Consequently, surgical decision-making must integrate anatomical complexity—specifically the vertical trajectory and volumetric involvement of the sphincter—with patient-specific factors such as prior surgical failures and systemic pathologies like Crohn's disease. Modern practice mandates a risk-stratified approach guided by preoperative imaging, prioritizing sphincter-preserving techniques whenever the functional risk of traditional fistulotomy is clinically unacceptable.

7. Surgical Management of Perianal Fistulas

Interventions are broadly divided into conventional obliterative procedures, such as fistulotomy, and more complex sphincter-preserving or minimally invasive techniques. Operative selection is an individualized process, guided by anatomical mapping to optimize the healing-to-continence ratio.

7.1 Conventional Techniques

7.1.1 Fistulotomy

Fistulotomy remains the gold standard for simple, low-lying fistulas, particularly intersphincteric and low transsphincteric tracts [26]. The procedure involves laying open the fistulous tract, allowing healing by secondary intention while eliminating the internal opening.

This technique achieves high healing rates, often exceeding 90% in appropriately selected cases [27, 28]. However, division of sphincter muscle limits its use in complex fistulas, where the risk of postoperative fecal incontinence becomes significant.

7.1.2 Seton Placement

Setons are commonly used to control infection and maintain drainage of fistulous tracts, particularly in complex or high fistulas [26]. Two main types are used:

1. Draining seton

A loose seton maintains tract patency and allows continuous drainage, preventing recurrent abscess formation while reducing local inflammation. It is frequently used as a temporary or staged intervention before definitive surgery.

2. Cutting seton

A tightened seton gradually divides the sphincter muscle while promoting fibrosis and tract closure. Although effective, this technique carries a risk of continence impairment, and its use has declined with the increasing availability of sphincter-preserving alternatives.

7.2 Sphincter-Preserving Techniques

7.2.1 Ligation of the Intersphincteric Fistula Tract (LIFT)

The LIFT procedure, originally described by Rojanasakul, has become a basis of sphincter-preserving surgery, with reported healing rates between 60% and 80% [29, 30]. Its primary advantage is the total preservation of the external sphincter by accessing the fistula through the intersphincteric plane. To address the commonest mode of failure—re-cannalization of the tract—modern refinements such as the LIFT-Plug and Bio-LIFT have been introduced.

In the LIFT-Plug technique, a bioprosthetic plug is inserted into the distal tract after ligation to act as a scaffold for tissue ingrowth and prevent fluid accumulation. Similarly, the Bio-LIFT approach involves placing a biological mesh within the intersphincteric space to create a reinforced physical barrier between the ligated ends of the tract [31]. While these hybrid strategies show promise in improving outcomes for high transsphincteric or recurrent fistulas, their use requires careful patient selection due to the increased cost of biological implants.

7.2.2. Endorectal Advancement Flap

The advancement flap procedure involves excision of the internal opening followed by coverage with a mobilized rectal mucosal flap. It is particularly useful for complex fistulas, recurrent disease, and patients at high risk of continence impairment. Although technically demanding, this method allows closure of the internal opening while preserving sphincter integrity.

7.2.3 Fistula Plugs and Biomaterials

Biologic fistula plugs and other biomaterials function as scaffolds that promote tissue integration and tract closure. Materials derived from porcine intestinal submucosa are most commonly used [32].

Adjunctive methods such as chemical ablation of the fistula tract have also been explored as minimally invasive treatment options. Various agents—including silver nitrate, phenol, fibrin glue, hydrogen peroxide, ethanol, and doxycycline—have been used with the aim of inducing local inflammation, fibrosis, or biological sealing of the tract to promote closure [33, 34, 35]. However, clinical outcomes have been inconsistent, and these approaches are

generally regarded as adjunctive or alternative therapies reserved for carefully selected cases rather than standard treatment options.

7.3 Minimally Invasive Techniques

7.3.1 FiLaC (Fistula Laser Closure)

FiLaC utilizes a radial-emitting laser probe to deliver thermal energy, causing shrinkage and obliteration of the fistula tract [36, 37]. This minimally invasive technique facilitates low postoperative morbidity and is ideally suited for day-case surgical management.

7.3.2 VAAFT (Video-Assisted Anal Fistula Treatment)

VAAFT employs a specialized fistuloscope that allows direct endoscopic visualization of the fistula tract, facilitating identification of the internal opening and targeted destruction of the tract [38, 39].

The ability to visualize secondary branches makes this approach particularly attractive for complex fistulas, although long-term success rates remain under evaluation [40].

Note: Recent technological advances have introduced Video-Assisted Anal Fistula Treatment (VAAFT) and Fistula Laser Closure (FiLaC), both designed to achieve tract obliteration with minimal tissue trauma. While these techniques offer the clear advantages of reduced postoperative pain, shorter recovery times, and high sphincter preservation, their long-term healing rates remain under rigorous evaluation compared to traditional methods.

Beyond clinical efficacy, the widespread adoption of these procedures is significantly influenced by economic and logistical factors. VAAFT requires a specialized fistuloscope system, whereas FiLaC relies on laser generators with radial-emitting probes; these technologies involve additional equipment and training requirements, which may limit their availability to high-volume, specialized colorectal centers [41]. Consequently, current international surveys suggest that only a minority of surgeons routinely perform these procedures. In contemporary practice, VAAFT and FiLaC are viewed as complementary, "precision-based" options for selected complex fistulas rather than universally accessible standard treatments, particularly in resource-constrained settings.

7.3.3 Stem Cell Therapy

Regenerative therapies using mesenchymal stem cells, particularly adipose-derived stem cells, have shown promising results in the treatment of Crohn’s disease–related fistulas [42, 43]. These therapies aim to promote tissue repair and modulate inflammation, offering a novel biologic approach for refractory disease.

Table 2: Selection of Surgical Techniques According to Fistula Complexity.

Fistula Type	Typical Characteristics	Preferred Surgical Options	Comments
Simple fistulas	Low intersphincteric or low transsphincteric tracts with minimal sphincter involvement and no secondary extensions	Fistulotomy	Considered the gold standard; high healing rates with minimal risk of continence impairment when appropriately selected
Complex fistulas	High transsphincteric, suprasphincteric, or extrasphincteric tracts; multiple branches or openings	LIFT, advancement flap, seton placement	Sphincter-preserving strategies are preferred to reduce risk of fecal incontinence
Recurrent fistulas	Prior failed surgery, scarred tissue planes	LIFT, advancement flap, minimally invasive techniques	Preoperative MRI recommended to delineate secondary tracts
Fistulas associated with systemic disease	Crohn's disease, tuberculosis, malignancy, radiation injury	Seton drainage combined with disease-specific therapy; selected sphincter-preserving procedures	Multidisciplinary management often required
Highly complex or branching fistulas	Horseshoe tracts, supralevator extension, multiple secondary branches	Combined or staged procedures; VAAFT, FiLaC in selected cases	Detailed imaging essential for surgical planning

8. Special Clinical Scenarios and Complex Presentations

Certain clinical phenotypes demand specialized management strategies to account for underlying systemic disease or high-risk anatomical configurations. In Crohn's disease, the therapeutic focus shifts toward a multidisciplinary paradigm, combining biological medical therapies with conservative surgical interventions [26, 44]. The primary surgical goal is the control of perianal sepsis, typically achieved through long-term draining seton placement, while avoiding aggressive fistulotomy that could lead to non-healing wounds or proctectomy.

In contrast, the management of recurrent fistulas necessitates a rigorous diagnostic reassessment to identify previously occult secondary tracts or inadequate prior drainage. High-resolution MRI mapping is indispensable in these cases to guide definitive secondary interventions. Similarly, horseshoe fistulas represent a significant technical challenge; these complex posterior tracts extend bilaterally into the ischioanal fossae and often involve the deep postanal space [17, 45]. Traditional management via the Hanley procedure involves the complete excision of the posterior midline tract with wide drainage of lateral extensions [46]. However, modern refinements, such as the modified Hanley procedure, utilize limited midline excision combined with staged drainage or flap-based repairs to achieve tract eradication while reducing the risk of sphincter injury [47].

Finally, anterior fistulas in female patients require a highly cautious, sphincter-preserving approach [48]. In this demographic, obliterative techniques that spare the external sphincter are prioritized to maintain functional integrity and quality of life.

9. Outcomes and Complications

The success of fistula surgery is measured by the dual metrics of definitive healing and functional preservation. Healing rates are largely dictated by anatomical complexity; while simple fistulotomy yields success rates exceeding 90%, complex or high transsphincteric tracts managed with sphincter-preserving techniques (LIFT, flaps, or laser) show more variable prognostic profiles. Recurrence remains the primary challenge, often precipitated by failure to localize the internal opening or incomplete eradication of occult secondary branches.

Fecal incontinence is the most debilitating postoperative complication, with risk levels directly proportional to the volume of sphincter muscle divided. This necessitates a precision-based approach, particularly for anterior tracts in females or patients with pre-existing weakness. Ultimately, the therapeutic goal extends beyond anatomical closure to the restoration of Quality of Life. A successful surgical 'cure' must achieve definitive tract healing without inducing a secondary functional morbidity more debilitating than the primary disease.

10. Postoperative Management and Functional Surveillance

The success of surgical intervention for perianal fistula is heavily dependent on a structured postoperative protocol aimed at optimizing wound healing and monitoring functional integrity. Immediate care focuses on pain modulation and local hygiene, typically through scheduled sitz baths and stool softeners to minimize mechanical trauma to the healing tissues. While routine antibiotic prophylaxis is generally not indicated, it remains reserved for patients with systemic signs of infection or extensive debridement.

Early surveillance, occurring within the first two weeks, is critical for the detection of secondary abscess formation or early recurrence, particularly following complex procedures such as advancement flaps or LIFT. For patients with draining setons, ensuring the patency and appropriate tension of the loop is paramount to maintaining controlled drainage of the intersphincteric space.

Long-term follow-up necessitates a multi-dimensional assessment of both anatomical and functional outcomes. Clinical evaluation at 4 to 12 weeks should be supplemented by objective imaging, such as 3D-EAUS or MRI, to confirm complete tract obliteration in complex or Crohn’s-associated cases. Crucially, functional outcomes must be quantified using validated scoring systems, such as the Wexner (Cleveland Clinic) Incontinence Score, to detect subtle changes in continence [49, 50].

11. Future Directions: Toward Precision Proctology

The management of perianal fistula is transitioning from traditional anatomical repair to a precision-based model. This shift is driven by the

integration of real-time intraoperative imaging—including high-resolution ultrasound and navigation-guided MRI—enabling the targeted eradication of occult secondary tracts while preserving sphincter integrity. Beyond structural mapping, advances in molecular biology are reshaping the understanding of fistula pathogenesis. Increasing evidence suggests that persistent tracts are influenced by local microbial dysbiosis and epithelial–mesenchymal transition (EMT), where epithelial cells acquire invasive mesenchymal characteristics that promote tract persistence. These mechanisms have stimulated therapeutic strategies aimed at restoring biological healing rather than simple excision. In parallel, regenerative approaches—including mesenchymal stem cell therapy, bio-engineered scaffolds, and cell-derived signaling mediators—are being investigated as sphincter-preserving solutions. The use of human amniotic membrane as an anti-inflammatory and regenerative framework has emerged as a promising experimental strategy, particularly when combined with mesenchymal stem cell–derived exosomes. These cell-free therapies aim to modulate epithelial–mesenchymal transition and local dysbiosis, thereby promoting tract healing without sphincter injury. Early clinical and preclinical studies suggest encouraging outcomes in complex and Crohn's-associated fistulas; however, evidence remains limited, and these approaches should be considered investigational pending validation in larger controlled trials [51, 52, 53]. In systemic conditions such as Crohn's disease, identifying specific inflammatory biomarkers may further enable personalized risk stratification and targeted biological therapy.

Finally, the integration of Artificial Intelligence (AI) and machine-learning algorithms is poised to transform preoperative planning and clinical decision-making [54, 55]. By analyzing large datasets of pelvic MRI images, surgical techniques, and long-term outcomes, AI-driven decision-support systems may assist surgeons in predicting recurrence risk and selecting the most appropriate operative strategy.

12. Strategic Decision Algorithms and Clinical Synthesis

The modern management of perianal fistula is governed by a structured, risk-stratified decision-making framework. This algorithm prioritizes the preservation of fecal continence without compromising the goal of definitive tract obliteration.

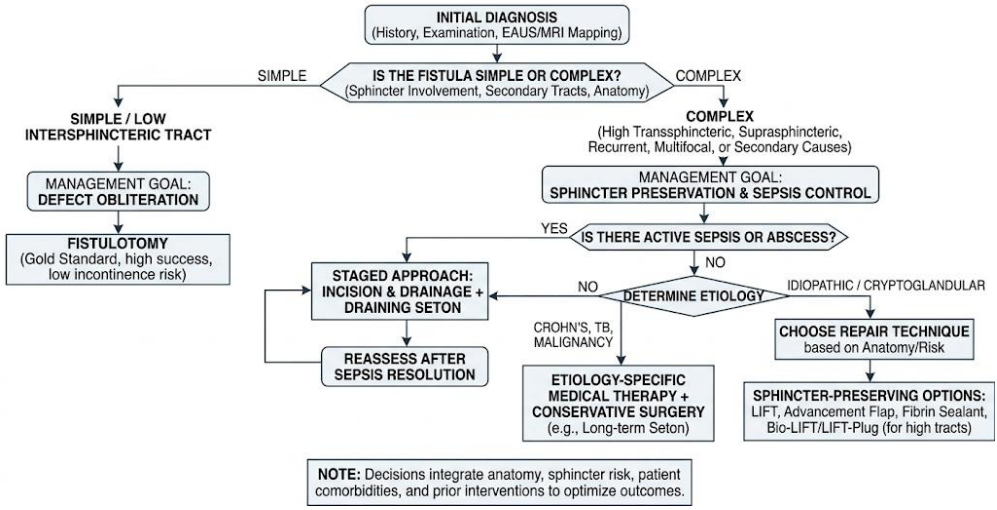
- Simple, Low-Lying Fistulas: For tracts involving minimal sphincter muscle, fistulotomy remains the gold standard, offering the highest success rates with negligible functional risk [2].
- Complex Presentations: High transsphincteric, suprasphincteric, or recurrent fistulas mandate a sphincter-preserving strategy. Techniques such as LIFT, endorectal advancement flaps, or

bioprosthetic seals are prioritized to avoid the morbidity of muscular division [56].

- Recurrent and Multifocal Disease: Management should be guided by MRI-mapped anatomy, often requiring a staged approach with draining setons to control sepsis before definitive repair [2].
- Secondary Etiologies: In cases of Crohn's disease, tuberculosis, or malignancy, surgery is secondary to etiology-specific medical therapy. Intervention in these scenarios is conservative, aiming for symptomatic control rather than aggressive tract eradication [57].

Ultimately, the integration of anatomical mapping, sphincter-risk assessment, and patient-specific comorbidities allows for a precision-based surgical plan that optimizes healing while minimizing the risk of recurrence.

STRATEGIC THERAPEUTIC ALGORITHM FOR PERIANAL FISTULA MANAGEMENT



13. Conclusion

Perianal fistulas remain a complex anorectal disorder that demands precise anatomical understanding and careful therapeutic planning. Contemporary management integrates accurate classification, advanced imaging, and sphincter-preserving techniques to balance healing with functional preservation. Emerging innovations, including minimally invasive procedures, regenerative therapies, and data-driven decision support, are progressively refining treatment strategies. This evolving paradigm underscores a shift toward tailored, patient-centered care focused on durable healing and preservation of continence.

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CHAPTER 7

Severity-Weighted Significant-Third Dental Indices: An Integrated Framework for Identifying the Least-Favorable Third of an Adult Sample

Necmi Namal[1]

[1] Faculty of Medicine, Department of Public Health, Istanbul Beykent University, Istanbul, Türkiye

1. Introduction

Dental epidemiology depends on summary measures that reduce complex tooth-level information to interpretable person-level and population-level indicators. This reduction is necessary for surveillance, comparison, planning, and evaluation, but it inevitably compresses information. The conventional Decayed, Missing, and Filled Teeth (DMFT) index remains one of the most familiar measures of cumulative caries experience. Its strengths are simplicity, historical continuity, and comparability. Its limitation is equally clear: teeth within a component are counted as units, so clinically different tooth states can contribute identically to the final count. Problems arising from the structure and analysis of DMF data have therefore long been discussed in dental epidemiology [1,2].

A second, distinct form of information loss occurs at the population level. A population mean can be informative about central tendency while saying little about the people concentrated at the unfavorable end of the distribution. This concern motivated the Significant Caries Index (SiC). Bratthall proposed ranking individuals by DMFT, selecting the one-third with the highest scores, and calculating the mean DMFT within that subgroup [3]. The SiC did not replace DMFT; rather, it changed the population summary so that a high-caries segment would remain visible. Subsequent work applied SiC across countries and areas and reinforced the usefulness of describing both the population mean and the high-burden tail [4].

The same ranking logic can be applied to a positively directed dental-status measure. The Filled and Sound Teeth (FS-T) index counts teeth that are sound or filled and retained, thereby providing a complementary perspective to DMFT [5]. Because higher FS-T values are favorable, the least-favorable segment is identified by ranking in the opposite direction. The Significant Filled and Sound Teeth (SFS-T) approach therefore focuses on the one-third with the lowest FS-T values [6]. This distinction is important: significant-third analysis is not inherently a disease index. It is a population-ranking operation whose direction depends on the meaning of the parent measure.

Severity weighting addresses a different problem. Instead of asking only how many teeth fall within broad categories, it allows ordered tooth states to contribute differently. In the present framework, W-DMFT, W-DM, and WFS-T are treated as pre-specified parent measures. Their scoring systems are not redeveloped in this chapter. The new question is what happens when the significant-third principle is applied after severity-sensitive person-level scores have been calculated. In other words, does severity information change the identity of the people classified within the least-favorable third?

The rationale for retaining ordered clinical information is also consistent with broader developments in caries epidemiology. Long-term changes in caries

occurrence and treatment patterns have altered the composition of conventional DMF experience [7]. Contemporary systems such as ICDAS/ICCMS, CAST, and PUFA demonstrate that lesion stage and clinical consequences can be recorded explicitly rather than compressed into a single affected-tooth category [8–10]. This approach is compatible with the modern understanding of dental caries as a dynamic disease process with clinically meaningful variation in stage and consequence [11].

2. Conceptual Rationale: Count, Severity, and Population Concentration

Three measurement dimensions should be separated conceptually. The first is count: how many teeth contribute to a conventional component or index. The second is severity composition: what ordered tooth states underlie those counts. The third is population concentration: which individuals occupy the least-favorable segment after person-level scores have been ranked. Conventional DMFT and FS-T primarily address the first dimension. W-DMFT, W-DM, and WFS-T introduce the second. SiC and SFS-T address the third. Severity-weighted significant-third measures combine the second and third dimensions while remaining anchored to their parent indices.

This separation prevents an important interpretive error. A significant-third derivative is not a new tooth-scoring system. It is calculated only after the parent index has been obtained for every individual. Consequently, S-WDMFT does not alter W-DMFT weights, S-WDM does not alter W-DM weights, and S-WFST does not alter WFS-T weights. The derivative changes only the ranking-and-selection step at population level.

The framework also preserves directionality. W-DMFT and W-DM are burden measures: larger values represent less favorable status. WFS-T is a retained-health measure: larger values represent more favorable status. The least-favorable third is therefore the upper third for W-DMFT and W-DM but the lower third for WFS-T. Treating these directions explicitly is essential for conceptual consistency.

3. Parent Weighted Measures

3.1 W-DMFT

The published W-DMFT conceptual model defines the index as the mean weighted score per assessed tooth [12]. Each tooth is assigned an ordered score: sound (S)=0; D1=1, D2=2, D3=3; F1=1, F2=2, F3=3; and missing (M)=3. For n assessed tooth positions:

$$\text{W-DMFT} = \Sigma w_i / n$$

With third molars excluded and a complete 28-tooth reference dentition, n=28. The theoretical range is 0–3. The 1–2–3 structure is an ordinal severity architecture: it should not be interpreted as proof that successive biological states

are separated by equal clinical intervals. The role of the weighting scheme is to retain ordered information that a simple affected-tooth count would otherwise collapse.

3.2 W-DM

W-DM isolates the decayed and missing components. D1=1, D2=2, D3=3, and M=3; all other states contribute 0. It is calculated as:

$$W\text{-}DM = \Sigma w_i / n$$

Its theoretical range is likewise 0–3, with higher values indicating a greater weighted burden attributable to decayed and missing teeth. Conceptually, W-DM is narrower than W-DMFT because restored teeth do not contribute to the score.

3.3 WFS-T

WFS-T is positively directed and represents retained dental status. Sound teeth and the most favorable restored state receive the maximum positive contribution, while progressively more structurally involved restorations receive lower positive contributions: S=3, F1=3, F2=2, F3=1, and D/M=0. The harmonized per-tooth formulation is:

$$WFS\text{-}T = \Sigma w_i / n$$

The theoretical range is 0–3, with higher values representing a more favorable retained dental state. An important conceptual point is that F1 receives a score of 1 in W-DMFT but 3 in WFS-T. This is not a contradiction. W-DMFT is negatively directed and asks how much weighted cumulative burden is represented by an affected tooth; WFS-T is positively directed and asks how much retained positive value is represented by the tooth. The same clinical category can therefore occupy different positions when the constructs themselves point in opposite directions.

4. Extending the Significant-Third Principle

The significant-third derivatives are defined by applying the same one-third population rule to the weighted parent measures. After all individual scores have been calculated, participants are ranked according to the relevant parent index. No new weights are introduced at this stage.

S-WDMFT = mean W-DMFT among the highest W-DMFT third

S-WDM = mean W-DM among the highest W-DM third

S-WFST = mean WFS-T among the lowest WFS-T third

The conventional comparison groups follow the same logic: SiC is the mean DMFT in the highest DMFT third, whereas SFS-T is the mean FS-T in the lowest FS-T third. The central analytical issue is not merely whether the subgroup means

differ; it is whether the identities of the individuals occupying these tails change when severity-sensitive ranking replaces conventional count-based ranking.

Table 1. Operational definition of conventional and weighted significant-third indices

Index	Parent index	Least-favorable third	n
SiC	DMFT	Highest DMFT scores	40
SFS-T	FS-T	Lowest FS-T scores	40
S-WDMFT	W-DMFT	Highest W-DMFT scores	40
S-WFST	WFS-T	Lowest WFS-T scores	40
S-WDM	W-DM	Highest W-DM scores	40

Note. The significant-third operation changes only the population-ranking and selection step. The scoring rules of the weighted parent indices remain unchanged.

5. Materials and Methods

5.1 Study design and empirical purpose

The empirical component was designed as a retrospective methodological illustration using existing anonymized adult dental records. Its purpose was not to redevelop W-DMFT, W-DM, or WFS-T and not to repeat an empirical comparison of weighted versus conventional parent scores. Instead, the analysis addressed a separate population-level question: when pre-specified severity-weighted scores are used for ranking, how does membership of the least-favorable third compare with membership defined by conventional indices?

5.2 Record selection and sample construction

Eligible records were from adults aged 18–64 years. A computer-generated sex-stratified random sample of 120 anonymized records was selected, comprising 60 men and 60 women. The random selection was fixed before the significant-third analyses and was not altered in response to the observed results. The mean age of the selected sample was 35.50 ± 13.09 years. Third molars were excluded, so each participant contributed up to 28 assessed tooth positions. Because 120 is exactly divisible by three, each significant-third group contained 40 participants.

The balanced sex allocation was a sampling design decision intended to ensure equal representation of men and women in this methodological illustration. It should not be interpreted as an estimate of the sex distribution in the source clinical population. Likewise, the sample was not intended to provide population prevalence estimates.

5.3 Tooth-level classification

Each assessed tooth occupied one mutually exclusive category: sound (S), decayed at one of three ordered levels (D1, D2, D3), filled/restored at one of three ordered levels (F1, F2, F3), or missing (M). D1–D3 represented increasing biological severity of decay. F1–F3 represented increasing structural involvement

among restored teeth, with F1 the most favorable restored state and F3 the greatest structural involvement within the operational classification.

5.4 Conventional indices

DMFT was calculated as D + M + F, with D comprising D1–D3 and F comprising F1–F3. FS-T was calculated as S + F. Higher DMFT indicates greater cumulative caries experience, whereas higher FS-T indicates a more favorable retained-dentition status.

5.5 Calculation of weighted parent indices

For each participant, W-DMFT, W-DM, and WFS-T were calculated using the scoring rules described in Section 3. All three were expressed on the harmonized 0–3 per-tooth scale. This common numerical range facilitates presentation but does not make the constructs interchangeable. W-DMFT and W-DM remain burden-oriented, whereas WFS-T remains positively directed.

5.6 Construction of significant-third groups

Participants were ranked separately for each parent measure. For DMFT, W-DMFT, and W-DM, ranking proceeded from highest to lowest and the first 40 individuals formed the least-favorable third. For FS-T and WFS-T, ranking proceeded from lowest to highest and the first 40 individuals formed the least-favorable third. SiC, SFS-T, S-WDMFT, S-WFST, and S-WDM were then calculated as the mean parent-index value within the corresponding 40-person group.

5.7 Membership overlap and reclassification

Worst-third membership was represented as a binary classification for each measure. Pairwise intersections were calculated for all five significant-third groups. Particular attention was given to the corresponding conventional-weighted comparisons SiC versus S-WDMFT and SFS-T versus S-WFST. Agreement beyond chance was summarized with Cohen's kappa. Pairwise and three-way intersections among S-WDMFT, S-WFST, and S-WDM were also calculated to identify the common core of individuals classified as least favorable across the three weighted perspectives.

Individuals who changed membership between conventional and corresponding weighted groups were examined descriptively at tooth level. For SiC versus S-WDMFT, D1–D3, F1–F3, and M profiles were inspected. For SFS-T versus S-WFST, particular attention was paid to the composition of sound and restored teeth. These case profiles were used to explain the mechanism of reclassification rather than to make individual clinical judgments.

5.8 Boundary ties and sensitivity analysis

Ties can occur at the cut-point separating the 40th and 41st ranked individuals. The primary analysis retained an exact group size of 40 for every significant-third measure using a fixed deterministic ordering. Because alternative allocations among individuals sharing the same boundary score can alter membership statistics without altering the defining index value, all permissible boundary allocations were examined in a sensitivity analysis. Ranges of pairwise overlap and kappa were recorded, together with the range of the three-way weighted intersection.

5.9 Statistical interpretation

Descriptive statistics were reported as mean ± standard deviation, median, and range where appropriate. Membership overlap was reported as counts and percentages. Cohen's kappa was used as a descriptive measure of agreement beyond chance for equal-sized binary least-favorable-third classifications. The analysis was not designed to establish clinical superiority, criterion validity, or predictive validity of the weighted parent indices. The primary outcome was population-tail membership and its reclassification after severity-sensitive ranking.

5.10 Ethical considerations

The empirical analysis used existing fully anonymized retrospective records and involved no participant contact. No identifying information was included in the analytical dataset. The final publication statement should follow the applicable institutional and national requirements governing research based exclusively on anonymized archival records. The work was conducted in accordance with the ethical principles applicable to retrospective human-data research.

6. Results

6.1 Distribution of parent and significant-third measures

In the 120-participant sample, mean DMFT was 8.858 ± 8.397 and mean FS-T was 23.067 ± 6.382. Mean W-DMFT was 0.728 ± 0.744, mean WFS-T was 2.404 ± 0.691, and mean W-DM was 0.521 ± 0.678. After selecting the least-favorable third, SiC was 19.125 ± 5.365 and SFS-T was 16.050 ± 6.606. The corresponding weighted derivatives were S-WDMFT 1.603 ± 0.615, S-WFST 1.632 ± 0.679, and S-WDM 1.266 ± 0.704 (Table 2).

Table 2. Scores in the total sample and in the least-favorable third

Measure	Total sample (n=120), mean ± SD	Least-favorable third (n=40), mean ± SD	Median	Range
DMFT / SiC	8.858 ± 8.397	19.125 ± 5.365	18	12–28
FS-T / SFS-T	23.067 ± 6.382	16.050 ± 6.606	18	0–23
W-DMFT / S-WDMFT	0.728 ± 0.744	1.603 ± 0.615	1.411	0.964–3.000
WFS-T / S-WFST	2.404 ± 0.691	1.632 ± 0.679	1.804	0–2.357
W-DM / S-WDM	0.521 ± 0.678	1.266 ± 0.704	1.071	0.536–3.000

Note. W-DMFT, WFS-T, and W-DM are expressed on a 0–3 per-tooth scale. Higher W-DMFT and W-DM values indicate greater burden; lower WFS-T values indicate less favorable retained dental status.

6.2 Conventional-weighted membership agreement

SiC and S-WDMFT shared 37 of their 40 members, corresponding to 92.5% overlap. SFS-T and S-WFST likewise shared 37 of 40 members (92.5%). For each of these two corresponding conventional-weighted comparisons, the primary Cohen's kappa was 0.888. Thus, severity-sensitive ranking retained most of the individuals identified by the conventional significant-third procedure while changing membership for a small boundary subgroup.

6.3 Pairwise overlap across all five significant-third groups

The complete membership matrix is shown in Table 3. SiC shared 32 participants with SFS-T, 37 with S-WDMFT, 33 with S-WFST, and 33 with S-WDM. SFS-T shared 34 participants with S-WDMFT, 37 with S-WFST, and 39 with S-WDM. The high SFS-T–S-WDM overlap reflects the close relationship between a low retained-tooth count and a high decayed/missing burden in this sample, while the incomplete overlap with W-DMFT and WFS-T shows that restoration composition can alter ranking.

Table 3. Pairwise membership overlap among conventional and weighted significant-third groups

	SiC	SFS-T	S-WDMFT	S-WFST	S-WDM
SiC	40	32	37	33	33
SFS-T	32	40	34	37	39
S-WDMFT	37	34	40	35	34
S-WFST	33	37	35	40	38
S-WDM	33	39	34	38	40

Note. Entries are numbers of common participants. Each significant-third group contains 40 participants.

6.4 Convergence among the three weighted significant-third measures

S-WDMFT and S-WFST shared 35 participants (87.5%), S-WDMFT and S-WDM shared 34 (85.0%), and S-WFST and S-WDM shared 38 (95.0%). Thirty-three participants belonged simultaneously to all three weighted least-favorable

thirds, corresponding to 82.5% of a 40-person significant-third group (Table 4). The weighted measures therefore identified a substantial common core without being interchangeable.

Table 4. Overlap among the three weighted significant-third indices

Comparison	Common participants (n)	Overlap within a 40-person group
S-WDMFT ∩ S-WFST	35	87.5%
S-WDMFT ∩ S-WDM	34	85.0%
S-WFST ∩ S-WDM	38	95.0%
S-WDMFT ∩ S-WFST ∩ S-WDM	33	82.5%

Note. The three-way intersection represents participants simultaneously classified within the least-favorable third by W-DMFT, WFS-T, and W-DM.

6.5 Reclassification between SiC and S-WDMFT

Three individuals were classified by SiC but not by S-WDMFT, and three entered S-WDMFT despite not being retained in the primary SiC group (Table 5). The profiles illustrate why count-based and severity-weighted ranking can diverge. Participant 84 had DMFT=12 but a W-DMFT of 0.643; the profile included eight F1 restorations, two F2 restorations, and two missing teeth. In contrast, participant 72 had a lower conventional DMFT of 11 but all 11 affected teeth were missing, producing W-DMFT=1.179. Participant 196 had DMFT=12 entirely from missing teeth and W-DMFT=1.286. Thus, conventional counts can rank individuals similarly or even less unfavorably despite markedly different weighted compositions.

Table 5. Participants reclassified between SiC and S-WDMFT at the significant-third boundary

Classification change	Patient	DMFT	D1/D2/D3	F1/F2/F3	M	W-DMFT
SiC only	77	13	0/0/0	6/1/1	5	0.929
SiC only	84	12	0/0/0	8/2/0	2	0.643
SiC only	172	12	0/0/0	6/0/0	6	0.857
S-WDMFT only	72	11	0/0/0	0/0/0	11	1.179
S-WDMFT only	160	11	0/0/0	3/0/0	8	0.964
S-WDMFT only	196	12	0/0/0	0/0/0	12	1.286

Note. Primary groups were fixed at exactly 40 participants. The profiles show the severity composition underlying conventional DMFT counts; some boundary membership can be tie-dependent.

6.6 Reclassification between SFS-T and S-WFST

The same pattern was visible from the retained-health perspective. Three individuals were classified by SFS-T only and three by S-WFST only in the primary allocation (Table 6). Participant 55 had FS-T=23, including 15 F1 restorations, and a WFS-T of 2.464. Because F1 receives the same positive contribution as a sound tooth in WFS-T, this restoration profile retained a high

weighted positive value. Participant 69 also had FS-T=23 but included seven F2 restorations and had WFS-T=2.214. Participant 127 had the higher conventional FS-T count of 24, but its mixture of five F1, three F2, and two F3 restorations yielded WFS-T=2.321. These examples show that the weighted positive measure can distinguish restoration composition that FS-T deliberately treats equally.

Table 6. Participants reclassified between SFS-T and S-WFST at the significant-third boundary

Classification change	Patient	FS-T	S	F1/F2/F3	WFS-T
SFS-T only	11	23	23	0/0/0	2.464
SFS-T only	55	23	8	15/0/0	2.464
SFS-T only	172	22	16	6/0/0	2.357
S-WFST only	69	23	12	4/7/0	2.214
S-WFST only	77	23	15	6/1/1	2.357
S-WFST only	127	24	14	5/3/2	2.321

Note. WFS-T is expressed on the harmonized 0–3 per-tooth scale. Lower values indicate a less favorable retained dental state. Some discordance at the cut-point may be tie-dependent.

6.7 Sensitivity to boundary ties

The principal overlap patterns remained stable when all permissible allocations among tied boundary scores were examined. SiC versus S-WDMFT overlap ranged from 37 to 38 participants. SFS-T versus S-WFST ranged from 36 to 39. For the weighted comparisons, S-WDMFT versus S-WFST ranged from 34 to 35, S-WDMFT versus S-WDM from 32 to 35, and S-WFST versus S-WDM from 36 to 38. The primary kappas were 0.888, 0.888, 0.813, 0.775, and 0.925, respectively. The three-way weighted intersection was 33 participants in the primary allocation and ranged from 31 to 33 across permissible tie allocations. The substantive conclusion—high convergence with selective reclassification—was therefore not dependent on one arbitrary boundary allocation.

7. Discussion

7.1 Main findings

This chapter applied the significant-third population-ranking principle to three pre-specified severity-weighted dental measures in a newly selected 120-record methodological sample. Four findings are central. First, corresponding conventional and weighted significant-third groups showed high but incomplete membership agreement: SiC–S-WDMFT and SFS-T–S-WFST each overlapped by 92.5%. Second, the three weighted derivatives identified a substantial common core, with 82.5% of a significant-third group shared by S-WDMFT, S-WFST, and S-WDM. Third, discordant cases were interpretable from the underlying tooth-state composition. Fourth, sensitivity analysis showed that boundary ties changed a small number of identities but did not overturn the overall pattern.

7.2 Why high agreement is expected—and why perfect agreement is not required

The weighted derivatives remain anchored to familiar dental constructs. W-DMFT retains D, M, and F information; W-DM retains D and M; WFS-T retains sound and filled teeth from a positive perspective. High agreement with conventional significant-third classifications is therefore expected and desirable. A severity-sensitive extension that identified a completely unrelated subgroup would raise concern that the construct had drifted too far from its parent measure.

Perfect agreement, however, would imply that the additional severity information never changes population ranking. The observed pattern lies between these extremes. Most individuals remain in the same least-favorable third, but a limited boundary subgroup is reclassified when tooth states that are equal under conventional counting receive different ordered contributions. This selective non-redundancy is the central empirical behavior that significant-third weighting is designed to reveal.

7.3 Reclassification is a composition effect, not a claim of clinical truth

The reclassified cases should be interpreted cautiously. When an individual with many F1 restorations moves out of a W-DMFT-defined tail, or an individual with many missing teeth moves into it, the result follows from the scoring architecture. It does not establish that one individual is clinically "worse" in every meaningful sense. Likewise, WFS-T's treatment of F1 as retaining the maximum positive contribution is a modelling choice aligned with the positive retained-health construct; it is not a claim that a restored tooth is biologically identical to an untouched sound tooth.

This distinction is essential for responsible use. Weighted indices are structured summaries. Their value depends on whether the ordered categories and weights prove useful, reproducible, and externally meaningful. The present analysis demonstrates how the ranking behaves under the specified scoring rules; it does not by itself validate those rules against symptoms, treatment complexity, chewing performance, prognosis, quality of life, or future tooth loss.

7.4 The population-tail perspective

The principal contribution of significant-third analysis is not the creation of a new diagnosis but the explicit description of distributional concentration. Bratthall's SiC was motivated by the recognition that mean DMFT can conceal a subgroup with substantially greater caries experience [3]. The same logic applies when the parent measure is severity weighted. Two populations could have similar mean W-DMFT yet differ in how weighted burden is concentrated among individuals. Conversely, a conventional SiC could be similar while the severity composition of the selected third differs. The derivative measures therefore create

a bridge between person-level severity composition and population-level concentration.

7.5 Complementarity of S-WDMFT, S-WDM, and S-WFST

The three weighted significant-third measures should not be expected to select exactly the same individuals because they represent related but distinct perspectives. S-WDMFT reflects cumulative weighted experience across decayed, missing, and restored states. S-WDM narrows attention to active/irreversible burden represented by decayed and missing states within the operational model. S-WFST asks a positive question about retained dental status. The 82.5% three-way intersection suggests a common unfavorable core, while the remaining non-overlap reflects differences in what each parent measure counts as informative.

The particularly high S-WFST–S-WDM overlap in this sample (95.0%) is consistent with the strong structural relationship between loss of retained teeth and accumulation of missing/decayed burden. Yet this relationship should not be assumed to generalize unchanged to populations with different treatment histories, restorative patterns, age structures, or access to care. A population with extensive restorative treatment could display a different relationship between W-DM, W-DMFT, and WFS-T.

7.6 Why the one-third threshold should be retained for now

The one-third cut-point is historically conventional rather than biologically privileged. Its value lies in continuity with SiC and SFS-T and in producing a subgroup large enough for stable description while still focusing on the unfavorable tail. The present design also has the practical advantage that n=120 yields an exact 40-person third. Alternative thresholds—such as the highest quartile, decile, or distribution-based cut-points—could be explored in future work, but doing so would answer a different methodological question. For initial development, retaining the established one-third operation makes the relationship to SiC transparent.

7.7 Boundary ties are part of the method, not a nuisance to hide

Any rank-based subgroup method can encounter equal scores at its cut-point. If the purpose is only to report the subgroup mean, alternative allocations of tied individuals may have little effect. If the purpose includes comparing individual membership, however, tie handling becomes methodologically important. Reporting an exact primary group together with a tie-sensitive range is therefore preferable to silently choosing one allocation. In the present sample, the main findings were robust to permissible tie reallocations, although some pairwise overlap values varied by several participants.

7.8 Relation to conventional oral-health surveillance

Standardized survey methods remain fundamental to oral-health surveillance and international comparability [13]. Severity-weighted significant-third measures should therefore be considered complementary analytical descriptors rather than substitutes for established surveillance indicators. Conventional DMFT, FS-T, SiC, and SFS-T remain readily interpretable and historically comparable. Weighted derivatives may add value when tooth-level severity categories are available and when the research question concerns composition within the unfavorable population tail.

A practical reporting strategy would be layered rather than substitutive: report the conventional population mean, the conventional significant-third measure, the weighted parent mean, and the weighted significant-third derivative. This preserves comparability while making both severity composition and concentration visible. Such reporting would also allow future studies to determine whether weighted tail measures explain outcomes beyond what is already captured by conventional indices.

8. Methodological Strengths

Several features strengthen the present methodological illustration. The sample was selected through a pre-specified sex-stratified random procedure rather than chosen to maximize a desired result. Equal numbers of men and women prevented sex imbalance from being built into the illustrative sample. The total n of 120 produced an exact third of 40, avoiding fractional group-size decisions. The parent weighted indices were calculated on a harmonized 0–3 per-tooth scale, and their scoring rules were kept fixed rather than modified after inspecting the sample. Finally, the analysis emphasized membership, reclassification, and tie sensitivity rather than treating mathematically expected score differences as evidence of superiority.

The use of both negatively directed burden measures and a positively directed retained-health measure is another strength. It demonstrates that the significant-third operation is independent of score direction provided that the ranking rule is specified correctly. This makes the framework potentially extensible to other oral-health indices whose favorable direction differs.

9. Limitations

The analysis is based on a clinical archival sample and is not population representative. The sex-balanced design was deliberate and therefore should not be used to infer the sex distribution of the underlying patient population. The sample size is adequate for a methodological illustration but is modest for subgroup analyses or external generalization. Replication in independent population-based datasets is required.

The significant-third derivatives inherit the assumptions and limitations of their parent weighted indices. In particular, the 1–2–3 scoring hierarchy is ordinal and parsimonious; it is not an empirically established interval scale. Assigning M the maximum burden score in W-DMFT and W-DM is operationally consistent with irreversible tooth loss but cannot establish the cause of every missing tooth from an archival odontogram. Similarly, assigning F1 the maximum positive WFS-T contribution is a construct-specific modelling decision.

The retrospective tooth-state categories also do not encompass every dimension of oral health. Periodontal status, pain, endodontic status, prosthetic replacement, occlusal function, patient-reported outcomes, and socioeconomic context are not represented in these indices. Therefore, the weighted significant-third groups should not be interpreted as comprehensive rankings of overall oral health or treatment priority.

Finally, membership agreement is partly influenced by the chosen one-third threshold and by ties near the boundary. The sensitivity analysis addresses the latter but cannot make the threshold itself biologically meaningful. Different cut-points could produce different membership patterns.

10. Validation and Research Agenda

The next stage should move beyond internal measurement behavior. First, reproducibility of D1–D3 and F1–F3 classification should be assessed through examiner calibration and inter-/intra-examiner reliability. Second, construct validity should be examined against independently measured indicators expected to relate to severity, such as treatment complexity, cumulative treatment need, structural loss, or clinically assessed disease consequences. Third, predictive validity should be tested longitudinally against outcomes such as future tooth loss, progression, retreatment, or functional deterioration.

Fourth, external replication should include populations with different age distributions, disease levels, restorative histories, and health-care systems. The composition of significant-third groups may differ markedly where untreated decay dominates compared with settings where restored teeth dominate. Fifth, sensitivity analyses should investigate alternative weighting functions. The current 1–2–3 structure is deliberately simple; future evidence may support non-linear weights or different treatment of missing teeth and restored states.

Sixth, the incremental information provided by weighted significant-third measures should be quantified. A useful question is not merely whether S-WDMFT differs from SiC, but whether it explains an external outcome after conventional DMFT and SiC are already known. The same applies to S-WFST relative to FS-T/SFS-T and to S-WDM relative to conventional D+M descriptors. Demonstrating incremental explanatory or predictive value would provide stronger justification for additional complexity.

Finally, future studies should pre-register sampling rules, tie handling, primary comparisons, and interpretation thresholds where feasible. This is particularly important for rank-based subgroup methods because small analytic choices at a boundary can affect individual membership even when group-level means remain stable.

11. Practical Calculation Workflow

For application in a new dataset, the procedure can be summarized in five steps. First, classify every assessed tooth using the required tooth-state categories. Second, calculate the conventional and weighted parent indices for every individual. Third, rank individuals separately for each parent measure in the direction that represents less favorable status. Fourth, select exactly one-third of the sample according to a pre-specified rule and calculate the mean parent score within that subgroup. Fifth, if membership comparisons are an objective, identify boundary ties and report a sensitivity range for alternative permissible allocations.

The crucial methodological safeguard is that the weighting system must be specified before the significant-third selection. The subgroup should never be used to tune the weights that subsequently define that same subgroup. Doing so would make the tail definition data-dependent and would compromise interpretability.

12. Conclusions

Severity-weighted significant-third measures provide a coherent way to combine two distinct epidemiological ideas: retaining ordered information about tooth-state severity and focusing attention on the least-favorable third of a population. In the present sex-stratified random sample of 120 adults, S-WDMFT and S-WFST each retained 92.5% of the membership identified by their conventional counterparts, while selective reclassification occurred in cases with different missing-tooth or restoration-severity compositions. S-WDMFT, S-WFST, and S-WDM shared an 82.5% common core, demonstrating substantial convergence without complete redundancy.

These findings should not be interpreted as proof that weighted indices are clinically superior to conventional measures. They demonstrate the behavior of a population-ranking extension under fixed scoring rules. The most appropriate current role of S-WDMFT, S-WDM, and S-WFST is therefore as complementary epidemiological descriptors that may reveal severity composition within the unfavorable population tail. Independent validation, reproducibility studies, longitudinal testing, and population-based replication are required before broader claims can be made.

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CHAPTER 7

Factors Affecting The Acceptance And Maintenance of Enteral Nutrition in Cancer Patients

***Selim Yalçın*[1]**

[1] Prof. Dr., Kırıkkale University Faculty of Medicine, Dept. of Internal Medicine, Medical Oncology Division, 0000-0003-1970-2849

1. Introduction

Cancer itself, the anatomical effect of the tumor, surgical interventions, radiotherapy, chemotherapy, and systemic inflammation can impair nutrient intake and nutrient utilization. Swallowing, chewing, salivary secretion, gastrointestinal passage, or oral intake are directly affected, especially in head and neck and upper gastrointestinal tract tumors. Nausea, vomiting, early satiety, pain, odynophagia, mucositis, taste alteration, and anorexia further limit oral intake. These processes can lead not only to weight loss but also to decreased treatment tolerance, increased risk of infection, loss of function, and decreased quality of life.

Enteral nutrition is the delivery of nutrients and fluids through an access route such as nasogastric, nasoenteric, gastrostomy, or jejunostomy, as long as the gastrointestinal system is functional. The clinical decision is broader than simply whether a tube can be technically placed. Insertion of a tube requires the patient to understand and accept the treatment plan, have the ability to implement it, tolerate symptoms, and, if possible, maintain home care. Similarly, tube removal should not be solely linked to reaching a certain weight or the reduction of acute symptoms. Safe and adequate oral intake, swallowing function, aspiration risk, tumor control, treatment toxicity, prognosis, and patient goals should be considered together. It has been reported that clinical compliance during tube feeding in head and neck cancer can remain at approximately half the level, and early feeding intervention can improve compliance. However, the effect of early intervention may be more pronounced in advanced-stage and high-risk patient groups; it cannot be directly generalized to patients with early-stage disease or those undergoing surgery only. Studies comparing prophylactic percutaneous endoscopic gastrostomy (PEG) with a reactive approach have shown selective benefits in short-term weight loss and quality of life, but have not demonstrated consistent superiority in survival, treatment discontinuation, and long-term outcomes. The aim of this review is to synthesize the factors influencing the acceptance of enteral feeding, adherence to the prescribed feeding volume, its maintenance throughout treatment, and its discontinuation at the appropriate time in the context of cancer. Clinical and disease-related variables; patient and psychosocial characteristics; tube, product, and application characteristics; The healthcare system and caregiver conditions were examined under separate headings. Subsequently, the reasons for dropping out, nursing and dietetic approaches, and clinical implications were discussed.

2. Conceptual Framework: Acceptance, Adherence, Continuity, and Discontinuation

When evaluating the outcomes of enteral nutrition, it is necessary to distinguish between four closely related but not identical concepts. Acceptance is when the patient finds the tube placement or enteral nutrition plan meaningful

and tolerable, voluntarily agrees to the recommendation, and participates in the decision-making process. Acceptance does not technically indicate that the prescribed volume has been reached. Adherence is the degree to which the prescribed volume, rate, frequency, water flushing, and care practices are actually performed. Continuity is when enteral nutrition continues as planned throughout the treatment period or the prescribed care period. Discontinuation may be clinically terminated due to the patient's request for tube removal, permanent cessation of tube use, discontinuation of the prescription, or transition to safe oral intake. This distinction is important. Not removing the tube does not mean that adequate nutrition is being provided; the patient may fall below the recommended volume even with a tube due to nausea, pain, depression, difficulty in care, or economic reasons. Conversely, tube removal is not always a failure. If oral intake has become safe and adequate, swallowing rehabilitation has progressed, and the tube is now more of a burden than a benefit, removal is the intended outcome of the treatment. Long-term tube dependence is not directly synonymous with discontinuation at the patient's request. Some patients may not want to use a tube, but it may need to be retained due to anatomical or functional limitations; others may continue to support oral intake using a tube. Acceptance and continuation are not the result of a single causal factor. Disease severity and initial oral intake capacity determine the need for a tube. As the need for a tube increases, the physical and social burden on the patient may also increase. This burden can be reduced through symptom control, education, caregiver support, and access to healthcare. Therefore, the clinical model should evaluate disease risk, patient values, and the feasibility of the plan on the same plane. In this context, the decision for enteral nutrition is based on answering the following four questions together:

1. Is the patient's gastrointestinal system and current clinical goals suitable for enteral nutrition?

2. For what reason and to what extent is oral intake insufficient; is the insufficiency temporary, progressive, or permanent?

3. Which access route, product, and method of administration is most suitable for the patient's symptoms, life, and care capacity? 4. Are the patient and caregiver able to create a joint plan by understanding the potential benefits, burdens, alternatives, and termination criteria?

3. Clinical and Disease-Related Factors

3.1. Head and Neck Cancer: Anatomical Obstruction, Stage, and Treatment Intensity

In head and neck cancer, the decision for enteral feeding is central to the tumor's impact on swallowing and oral intake. Oropharyngeal, hypopharyngeal, and laryngeal locations can disrupt the anatomical integrity and coordination of

the tongue, pharynx, larynx, and esophageal inlet. Large tumor burden, advanced T or N stage, bilateral neck irradiation, and the combined application of chemoradiotherapy increase swallowing failure both initially and during treatment.

In an observational study examining tube adherence in head and neck cancer, the high-risk group consisted mostly of patients with oropharyngeal tumors, stage IV, and receiving chemoradiotherapy. Clinical tube feeding adherence remained at approximately 48%, while adherence increased from 38% to 58% with early feeding intervention. This result suggests that initiating nutritional support only after weight loss may create a missed opportunity. However, the advanced stage and high risk of the sample limit the generalization of the findings to patients with earlier stage disease or those who only received surgical treatment. The timing of symptoms during treatment is also important. In the early stages, anorexia and taste changes may be prominent. In the following weeks, odynophagia, mucositis, dry mouth, and swallowing pain more significantly limit oral intake. Nausea, vomiting, early satiety, delays in hospitalization, and prescription changes can prevent reaching the recommended volume via intubation. Therefore, when compliance is low, it should not be assumed that the patient is unwilling; the timing and severity of the symptom, the medications used, the position of the intubation, the rate of feeding, and the continuity of the care plan should be examined together.

3.2. Prophylactic or Reactive Intubation Decision

A prophylactic intubation is a tube placed before clinical deterioration occurs, anticipating that oral intake will be impaired during treatment. In a reactive approach, the tube is inserted after insufficient intake or significant weight loss has occurred. A prophylactic approach can reduce the risk of emergency intubation and treatment interruption in high-risk patients; however, it can raise concerns such as unnecessary intubation, procedural complications, tube dependence, or decreased swallowing ability. Onset dysphagia, body mass index below 18.5, and hypopharyngeal tumors were significantly associated with prophylactic gastrostomy. High T/N grade, low performance status, bilateral neck irradiation, need for adapted oral consistency, and non-compliance with swallowing exercises were also reported risk determinants. These findings suggest that the decision for prophylactic tube insertion should not be based solely on weight or body mass index. Swallowing function, rehabilitation capacity, and planned radiotherapy should also be considered. In a systematic review including five studies and three randomized controlled trials, prophylactic PEG reduced critical weight loss by more than 10% in the short term and improved short-term quality of life. However, no timing effect was shown on other nutritional outcomes, treatment interruption, disease-free survival, and overall survival. The certainty of evidence was low for most outcomes, and

moderate for nutritional status and treatment interruption. In a single-center prospective comparison, weight loss during treatment was found to be lower in the prophylactic PEG group compared to the reactive or PEG-free group, but the non-randomization of the study retains the possibility of selection bias.

These results do not make the prophylactic approach routinely necessary or generally unnecessary. A more appropriate interpretation is that a prophylactic approach may provide selective benefit when factors such as initial dysphagia, low nutritional reserve, advanced stage, intensive chemoradiotherapy, expected long-term swallowing impairment, and limited care support are present. In low-risk patients with good oral intake, a reactive approach with monitoring may be reasonable. The decision should be made through an open benefit-risk discussion between the clinical team and the patient.

3.3. Tube dependence, radiotherapy dose, and swallowing rehabilitation

As important as maintaining enteral feeding is knowing when to reduce or discontinue it. In a prospective study of 61 patients with head and neck cancer, tube dependence was reported in 46.5% at six months and 31.5% at twelve months. Dose of 50 Gy or higher to at least 50% of the larynx was an independent predictor of long-term PEG dependence at six and twelfth months. This finding suggests that tube removal cannot be explained solely by weight stability. Late fibrosis due to radiotherapy, laryngeal sensory loss, aspiration, and impaired coordination of swallowing muscles may persist.

Completely discontinuing safe oral intake after tube placement may increase functional decline, especially in patients where preserving swallowing function is important. However, maintaining oral intake is not safe for every patient. The speech and swallowing therapist, in collaboration with the nutrition team, should regulate consistency, portioning, aspiration risk, and swallowing exercises, Tube removal

4. Patient and Psychosocial Factors

4.1. Information, Disease Perception, and Meaning of Decision

Even when the technical benefits of a feeding tube are explained to the patient, acceptance does not occur automatically. The patient's explanation of cancer and nutritional deficiency, the symbolic meaning of the tube in relation to disease progression, fear of dependence, and expectations regarding treatment benefits influence the decision. Systematic reviews of chronic disease literature have found a relationship between disease perception, health literacy, cultural or religious beliefs, self-efficacy, and treatment knowledge and adherence; however, the direction of the relationship has varied among studies. This evidence is not directly related to cancer enteral nutrition, but supports the idea that acceptance interviews cannot be conducted solely as information transfer. The patient should be told what problems the tube can solve, what problems it

cannot solve, what alternatives are available, and when it will be re-evaluated. Successful education is validated not by the patient repeating what is said, but by their ability to demonstrate what they will do in their own life. For example, the patient's skills in flushing the tube with water, not changing the feeding rate, knowing the route of intervention in case of obstruction or vomiting, and explaining the order in which medications are administered through the tube should be observed.

4.2. Body Image, Independence, and Social Life

The tube can affect body image as a visible sign of cancer and treatment. Body image anxiety was reported in 68% of head and neck cancer patients at diagnosis, and some level of anxiety was found in 89% of patients evaluated at three months after treatment. Body image anxiety may be associated with physical symptom burden, depression, social relationship and privacy issues, loss of self-esteem, and decreased quality of life. Especially in patients with treatment scars on the neck or face, the tube may increase the feeling of not being able to control the body.

The visibility of the nasogastric tube may create shame or anxiety during conversation, bathing, going out, and social encounters. Although the PEG is less visible, it can create stoma care, clothing choices, pain, and feelings of dependence. Although patients may initially experience reluctance and fear, they may find the tube useful over time. A qualitative systematic review has shown that physical discomfort, social life limitations, new care responsibilities, and acceptance that develops over time occur together. Therefore, initial refusal does not mean that the decision is definitively closed; the assessment may change with reconsideration and improvement of symptoms. The goal of independence should be supported by protecting the tasks the patient can safely perform, not by the caregiver replacing the patient. If possible, the patient should participate in skills such as preparing products, flushing the tube, positioning, or keeping daily records. If independence is not possible, the care plan should be designed to protect the patient's privacy and choices.

4.3. Anxiety, Depression, and Quality of Life

During cancer treatment, depression and anxiety may not manifest as tube refusal or reduced feeding volume; they may present as missed appointments, procrastination, sleep disturbances, social withdrawal, and fatigue. Longitudinal systematic reviews have shown that lower quality of life can be seen before the onset of depression or anxiety, further decrease during mood disorders, and remain below healthy comparison groups even after recovery. Since the relationship may be observational and bidirectional, enteral feeding should not be assumed to be the sole cause of non-compliance.

At the tube acceptance interview, the patient's anxiety, sleep status, social functioning, and signs of hopelessness regarding treatment can be assessed using brief screening tools. Psycho-oncology, psychiatry, or social work support should be provided as needed. Psychosocial interventions for adults newly diagnosed with chronic illnesses are promising in terms of acceptance, coping, and managing uncertainty; however, some studies are small, heterogeneous, and based on non-standard results. Therefore, psychosocial intervention should be planned as a complement to, not a replacement for, enteral nutrition education.

4.4. Family, Culture, and Joint Decision-Making

In home-based enteral nutrition, the family is often responsible for implementing the plan. Family members can integrate the illness into daily life, organize the care environment, and facilitate the continuation of treatment. A qualitative systematic review highlighted the functions of family support in maintaining solidarity, normalizing the illness, and integrating self-management into daily contexts. However, the family role can also create problems such as caregiving burden, conflict, reduced privacy, or overshadowing the patient's autonomy. The caregiver should not be the decision-maker for the patient; they should be recognized as a partner in implementing the plan at the patient's request. Culture can influence acceptance through dietary preferences, family roles, religious practices, attitudes toward traditional treatments, and disease descriptions. A mixed systematic review on preserving cultural identity examines food preferences, family support, structural barriers, and non-Western disease perspectives.

5. Product, Tube and Administration Factors

5.1. Volume, Rate, Modality and Gastrointestinal Tolerance

Maintaining enteral nutrition depends more on the patient's ability to tolerate the product than on its prescription. Reflux, vomiting, diarrhea, constipation, gas, abdominal distension and pain may be product-related; however, medications, infection, tube migration, obstruction, fluid deficiency, fiber deficiency, product concentration, temperature and infusion rate can also cause the same symptoms. Therefore, product intolerance should not be concluded from a single symptom. The ESPEN home enteral nutrition guideline defines bolus administration via gastric tube as dividing the total volume into four to six meals throughout the day, with each meal of approximately 200–400 mL given over 15–60 minutes; these values are not rigid prescriptions but practical ranges to be adapted to tolerance. In jejunal feeding, continuous and low-rate pump administration is often more suitable; bolus administration may increase the risk of abdominal discomfort and dumping. A pump may be useful in patients with tolerance issues or high energy requirements at low volumes; Bolus administration can facilitate daily life and independence from pump connection.

When vomiting and reflux occur, the patient should be kept upright at least 30–45 degrees, and the tube position and feeding rate should be reviewed. In distension and pain, constipation, air ingress, medications, and mechanical obstruction should be evaluated. In diarrhea, hydration-electrolyte balance, antibiotics or sorbitol-containing medications, infection, hygiene, formula, and rate should be examined. Fiber-containing formulas may reduce diarrhea in adults receiving enteral feeding in the hospital; the risk ratio was found to be 0.64 in a meta-analysis, but fiber types and patient groups are heterogeneous. Therefore, fiber should not be used as an automatic solution in mechanical obstruction or severe motility disorder.

5.2. Tube care and the impact of complications on acceptance

Obstruction, tube dislodgement or displacement, stoma infection, and pain directly affect the patient's willingness to maintain the tube. ESPEN recommends flushing with at least 30 mL of water before and after bolus, flushing approximately every four hours during continuous infusion, and administering medications separately if possible. The use of cola or empirical bicarbonate for tube flushing should not be considered a routine approach due to contamination and safety concerns.

Integrative reviews of nasogastric or nasoenteric tubes have reported obstruction, misplacement, aspiration, esophageal-pharyngeal damage, pressure ulcers, perforation, and unplanned removal. Obstruction rates ranged from 11–21% in different studies, nasal pressure ulcers due to fixation were 25.2%, and airway misplacement was approximately 2–3% in some series. These rates, coming from diverse clinical settings, should not be directly applied to cancer patients living at home; however, they highlight the importance of practical training and proper validation protocols.

In PEG or gastrojejunostomy, the stoma surroundings should be monitored daily; Bleeding, pain, erythema, leakage, induration, fever, and granulation should be evaluated. After tract healing, rotating and moving the tube as recommended can help prevent buried-bumper syndrome. The patient and caregiver should be clearly informed that emergency attention is required in case of tube displacement, respiratory distress during feeding, severe abdominal pain, persistent vomiting, fever, bleeding, or inability to flush the tube.

5.3. Home Administration Burden and Caregiver Capacity

Home enteral feeding, while technically simple, requires formula preparation, storage, timely administration, water flushing, medication management, and symptom monitoring. In a qualitative systematic review, caregivers reported difficulties with formula preparation, tube problems, daily administration, and the socio-emotional impacts of care; education, accessible professional support, and communication during the transition from hospital to home were highlighted.

This burden varies with the patient's functional independence, cognitive status, number of caregivers, and home environment. In a multicenter study examining the quality of life of caregivers receiving enteral nutrition at home, 19% of caregivers rated their quality of life as poor, 57% reported depression, and 51.2% stated they were dissatisfied with their financial situation. These findings demonstrate that tube and product selection cannot be based solely on clinical tolerance. The plan should consider pump, consumables, transportation, home nursing, telephone counseling, rest opportunities, and economic support.

6. Healthcare system and caregiver-related factors

6.1. Continuity of discharge, education, and follow-up

When enteral nutrition is initiated in the hospital and continued at home, the transition at discharge represents the most vulnerable point in care. Prior to discharge, details regarding the stable prescription, product and fluid requirements, tube placement, administration rate, stoma or nasal care, medication administration, signs of complications, and points of contact must be explained both verbally and in writing. The competence of the patient and caregiver should be verified not merely through verbal explanation, but through demonstrations and "teach-back" (return demonstration) sessions.

A Cochrane review examining individualized discharge plans found that tailoring the plan to the patient's needs reduced the length of hospital stay and readmission rates over a period of approximately three months. Interventions combining counseling, written and visual materials, information adapted for health literacy, and verification of understanding have also been shown to reduce readmissions while improving adherence and patient satisfaction. Although this evidence is not specific to enteral nutrition in cancer patients, it supports the clinical rationale for a structured transition plan for tube-based therapy.

Studies on nurse-led transitional care have shown that post-discharge telephone follow-up and self-management education reduce readmissions and emergency department visits. In the context of enteral nutrition, telephone consultations can be used for the early detection of issues regarding weight, hydration, vomiting, diarrhea, constipation, stoma status, tube occlusion, product access, and caregiver burden. Follow-up should not merely consist of a helpline accessed only when problems arise; rather, it should be a planned process with a designated responsible party during the initial days and weeks.

Continuity of dietitian recommendations is not always maintained after discharge. In the More-2-Eat study, only 42% of those who completed the post-discharge interview reported receiving nutritional advice, and 65% of those who received such advice adhered to it. This finding suggests that dietitian consultation within the hospital is not sufficient on its own; rather, goals need to

be documented in writing, practical training provided to the caregiver, and follow-up maintained at home or via tele-dietitian services.

6.2. Multidisciplinary Team and Communication

The enteral nutrition plan should be shared among the oncologist, surgeon, radiation oncologist, nurse, dietitian, speech and swallowing therapist, pharmacist, social worker, and psycho-oncologist as needed. Everyone in this team should share the same goal: why was the tube inserted, what volume is being targeted, how will oral intake be maintained, in which symptoms will the plan change, and under what conditions will the tube be removed?

Continuity between service areas is ensured by the timely transfer of accurate information, the presence of a responsible team or person known to the patient, and the identification of the owner of the follow-up plan. Loss of information, repeated training, conflicting recommendations, or ambiguity of referral responsibility can reduce patient trust and compliance. Healthcare professionals providing patient-specific information, showing empathy, and creating a shared plan are positively related to self-management; authoritarian communication, on the other hand, can weaken the sense of empowerment and agency. 6.3. Access, Cost, and Inequality

Maintaining enteral nutrition; Product reimbursement coverage is related to the supply of tubes and pumps, stoma materials, home health care, transportation, and access to healthcare facilities. A systematic review examining the cost of home care noted that home care can be less expensive or cost-effective than hospital care in many scenarios, but the household burden can remain invisible when caregiver free time, transportation, and out-of-pocket expenses are not taken into account. Therefore, the economic assessment should not be reduced solely to the expenditure of the health system.

Referral for home health care does not mean equal access to the service. A large Medicare data study showed racial, ethnic, and socioeconomic differences in the use of high-quality home health agencies; a significant portion of the differences were found to be related to neighborhood-level characteristics. In cancer patients, language, housing conditions, internet access, living alone, transportation, and social security should be assessed pre-discharge. It should not be assumed that the same educational material is equally applicable to every patient.

7. Reasons for Discontinuing Enteral Feeding and Re-evaluation

Discontinuing enteral feeding can occur in four different ways. First, the patient may reduce the use of the tube or request its removal because they cannot physically or psychologically accept it. Second, symptoms or complications may prevent reaching the prescribed volume. Third, caregiver burden, cost, or access to services may impair sustainability. Fourth, the clinical team may remove the

tube in a planned manner because safe and adequate oral intake has returned. When these pathways are recorded without separating them, failure is confused with appropriate treatment termination. Before determining which aspect of the tube is unacceptable to the patient, it should be determined. If there is pain, stoma infection, nasal discomfort, nausea, visibility, body image, or fear of care, a targeted solution can be attempted. The product and rate can be adjusted, the tube type can be re-evaluated, pain and nausea can be treated, and psychosocial support can be provided. However, if the patient has the capacity to make decisions, a persistent refusal made with sufficient information should not be labeled solely as non-compliance.

Weight stability alone is not sufficient for tube removal. Whether oral intake meets energy, protein, fluid, and medication requirements; whether swallowing is safe; Aspiration, cough, pneumonia, or significant residual dysphagia should be assessed. If the risk of insufficiency is high after tube removal, close monitoring and re-access planning should be implemented as needed. In patients undergoing swallowing therapy, the tube may be used as a temporary safety net to support rehabilitation rather than completely replacing oral intake. The framework for enteral feeding decisions in palliative care should be established even more carefully. Automatic tube continuation is not mandatory in cases of advanced cancer, near-end of life, progressive organ failure, or significant symptom burden. Expected benefits may include reduced hunger or thirst, medication or fluid administration, support for a patient-focused goal, and meaningfulness for the family. Potential harms include aspiration, diarrhea, edema, stoma problems, entrapment, hospital admissions, and care burden. The decision should be made jointly with the caregiver and team, taking into account the patient's capacity and previously stated goals. It has been reported that there is no reliable evidence that PEG improves survival or quality of life in advanced dementia, and some harms may occur. This finding should not be directly applied to cancer patients, but it demonstrates the need for caution against the assumption that the tube will be beneficial in every case. Similarly, it is recommended that gastrostomy decisions be discussed beforehand, considering the quality of life, care burden, symptoms, and future termination options.

8. Nursing and Dietetic Approaches that Increase Acceptance and Continuity

8.1. Risk-Based and Step-by-Step Assessment

The initial assessment should include weight and body mass index, involuntary weight loss, initial dysphagia, percentage of oral intake met, tumor location, stage, treatment intensity, performance status, swallowing test, comorbidities, cognitive status, mental symptoms, caregiver, and financial resources. This assessment should not be a one-time event but repeated at treatment milestones. The rapid change in symptoms, especially during

radiotherapy, necessitates dynamic updating of the prescription and implementation goals.

Risk stratification can prevent delayed reactive tube placement in high-risk patients. Conversely, routine prophylactic tube placement in low-risk patients should be carefully considered due to the workload and the possibility of long-term dependence. The decision should be tied to the patient's choice and the swallowing rehabilitation plan, to the extent permitted by the protocol.

8.2. Collaborative Decision-Making and Education

The educational interview should begin by asking about the patient's current knowledge and concerns. Nurses and dietitians should explain the purpose of the intubation, expected duration of use, alternative access routes, the impact of the intubation on life, complications, and removal criteria in simple language. Benefits and risks should be explained not in absolute terms, but with frequencies and uncertainties where possible. The patient's goals, such as continuing treatment, staying home, maintaining mealtimes with family, or maintaining speech and social life, should be included in the prescription design.

An approach that strengthens health literacy is not limited to simplifying written information. The patient should be expected to describe the plan in their own words, demonstrate the procedure, and express their questions. Collaborative decision-making does not mean that every option is equally appropriate; it means choosing the option that aligns with the patient's values from among clinically safe options.

8.3. Practical skills, symptom management, and record keeping

Before discharge, the patient and caregiver should demonstrate practical skills such as hand hygiene, positioning, product storage, volume and rate adjustment, rinsing with water, medication administration, stoma or nasal care, alarm and pump problems, and emergency sign indications. These skills can be reinforced with short videos, illustrated instructions, follow-up calls by phone, and home visits.

The dietitian should gradually increase the energy-protein target according to the patient's tolerance; explain how fluids and medications will be aligned with the enteral plan. A pre-determined action plan should be in place for diarrhea, constipation, vomiting, and distension. Medications, infection, rate, volume, fluid intake, tube position, and hygiene should be assessed before changing products. Recording the patient's daily feeding volume, oral intake, symptoms, stool status, weight, and referral needs facilitates communication with the team.

8.4. Multidisciplinary and Continuous Monitoring

In a multidisciplinary team, the name of the responsible coordinator should be given to the patient, a telephone or digital follow-up channel should be

determined, and the plan should be communicated to all healthcare professionals. Systematic reviews examining home enteral nutrition services show that structured education and multidisciplinary monitoring can increase knowledge levels and reduce tube obstruction and care problems; however, the effects are inconsistent and the quality of evidence is low in cases of hospitalization or unplanned admission. Therefore, follow-up should be designed not only to reduce the number of referrals, but also to monitor symptom burden, quality of life, caregiver trust, actual adherence to prescription, and patient preferences.

A multicenter study using personalized nutritional education, tube and complication management, and regular phone calls together reported improvement in nutritional status at six months and a reduction in some tolerance issues between three and six months. Since there was no control group, the results cannot be attributed solely to education; however, it suggests that hands-on training and regular contact is a clinically usable model. Video calling added to monthly home visits in frail elderly patients reduced metabolic and gastrointestinal complications; however, it did not show a difference in mechanical complications, referrals, or hospitalizations. Technology should be considered as a complementary tool in the appropriate patient, not a replacement for face-to-face access.

8.5. Supporting the Caregiver

Caregiver assessment should include the individual's health status, learning capacity, time and workload, sleep, depression, economic resources, other responsibilities at home, and contact persons in case of crisis. The entire responsibility should not be left to one person; if possible, task sharing and a rest plan should be established within the family. The nurse should observe the caregiver's actions and assess their safety using non-accusatory language.

The care burden has physical, emotional, social, and financial dimensions. Social service support, temporary relief services, home nursing, economic assistance, and psychological counseling should be added to the nutritional plan when needed. If the caregiver's capacity indicates that the plan is unrealistic, a lower caregiver burden, access route, pump, or monitoring **9. Clinical Implications**

model should be discussed.

A practical approach to making enteral nutrition decisions in cancer patients can be summarized with the following principles:

•The tube decision should be based on a combined assessment of tumor anatomy, dysphagia, stage, planned treatment, initial nutritional status, performance, and care capacity. •Prophylactic PEG may reduce short-term weight loss in high-risk head and neck cancer patients; however, it should not be considered a routine standard for all patients. •Failure to reach the prescribed

volume should be examined not as patient reluctance, but as a combined result of symptoms, tube, product, procedure, and continuity of healthcare. •Tube type selection should be individualized based on expected duration, anatomical suitability, aspiration risk, procedural risk, social visibility, caregiver skill, and patient preferences. •Safe oral intake and swallowing rehabilitation should be maintained while tube use; the decision to remove the tube should be linked to criteria of safe and adequate oral intake and functional recovery. •Enteral nutrition in palliative care should not be automatically continued solely on the assumption of prolonging life; symptom relief, quality of life, caregiver burden, patient goals, and risks should be considered together. •Nursing and dietetic monitoring should include hands-on training, feedback, symptom management, scheduled telephone or home monitoring, caregiver support, and a single coordination channel. •Reasons for adherence and discontinuation should be recorded in a standardized manner. Prescribed and delivered volume, involuntary tube removal, patient-requested discontinuation, discontinuation due to complications, transition to safe oral intake, and caregiver burden should be reported as separate outcomes. •In clinical trials, tumor type, HPV status, stage, radiotherapy dose, tube type, feeding protocol, patient preference, caregiver burden, and long-term swallowing function should be measured together.

Conclusion

The acceptance and maintenance of enteral nutrition in cancer patients is the result of a much broader interaction than simply the presence of the tube. Tumor anatomical obstruction and disease stage, initial dysphagia, treatment intensity, late swallowing disorder due to radiotherapy, and symptom burden determine the clinical need. The patient's knowledge and beliefs, body image, sense of independence, anxiety, quality of life, family, and cultural context influence how this need is understood and maintained in daily life. Tube type, product tolerance, volume and rate, complications, caregiver skills, cost, and access to healthcare also determine the real-life applicability of the prescribed support. The most appropriate approach is an individualized model that identifies high-risk patients early, avoids unnecessary procedures in low-risk patients, and allows for reassessment throughout treatment. Nurse and dietitian-led hands-on education, symptom-focused adjustment, swallowing rehabilitation, caregiver support, and planned transition monitoring can strengthen acceptance and retention. However, the tube should not be automatically maintained or removed solely based on weight stability in all cases. Especially in palliative care, the decision should be made collaboratively, considering the patient's life goals, symptom relief, functionality, caregiver burden, and the potential benefit-risk balance of the treatment. Future research should monitor patient-reported acceptance, actual volume compliance, caregiver burden, safe tube removal criteria, and long-term swallowing outcomes using cancer-specific and standardized methods.

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CHAPTER 8

Intracapsular versus Extracapsular Tonsil Surgery in Children: Current Evidence, Outcomes, and Controversies

***Malik Afifoğlu*[1]**

[1] Department of ENT, Bursa City Hospital, Bursa, Türkiye,
ORCID: 0000-0001-5938-3294

1. Introduction

Pediatric tonsil surgery has moved from a relatively simple question whether the tonsils should be removed to a more nuanced one: how much tonsillar tissue needs to be removed to achieve the therapeutic objective with the least morbidity Extracapsular tonsillectomy, in which the palatine tonsil and its capsule are removed by dissection in the peritonsillar plane, remains the reference operation and offers the most definitive elimination of tonsillar tissue. Intracapsular tonsil surgery, including tonsillotomy and near total intracapsular tonsillectomy, intentionally preserves the capsule and a variable rim of lymphoid tissue. Its principal rationale is to avoid direct exposure of the pharyngeal musculature and deeper neurovascular structures. Contemporary comparative evidence consistently associates intracapsular surgery with less postoperative pain, less analgesic use, earlier return to diet and activity, and lower postoperative hemorrhage rates (Hurly et al., 2025; Lao, Jian, Ge, & Wu, 2025).

The strongest evidence for intracapsular surgery is in children undergoing adenotonsillar surgery for obstructive sleep disordered breathing (oSDB) or obstructive sleep apnea (OSA). A 2023 systematic review of plasma ablation intracapsular surgery found that children became pain free approximately 4.2 days earlier, discontinued analgesia 4.1 days earlier, resumed normal diet 3.5 days earlier, and returned to normal activity 2.8 days earlier than children undergoing total tonsillectomy; the pooled risk of postoperative hemorrhage was also lower (risk ratio [RR] 0.36, 95% confidence interval [CI] 0.16–0.81). The larger 2025 meta analysis of 32 studies and 9,430 children reached a similar conclusion: tonsillotomy shortened time to normal diet by 2.49 days, reduced duration of analgesic requirement by 3.19 days, and reduced secondary bleeding (RR 0.33, 95% CI 0.23–0.47), while improvement in OSA-18 was not significantly different from total tonsillectomy (Lao et al., 2025).

The principal counterweight is long term failure related to residual lymphoid tissue. Tonsillar regrowth, recurrent obstruction, recurrent infection, and completion tonsillectomy are genuine outcomes, although their frequency varies markedly according to patient age, operative definition, surgical depth, instrument, institutional experience, and duration of follow up. Large contemporary series report revision rates ranging from approximately 1–3% in many established intracapsular programs, but higher rates occur in selected cohorts and with longer surveillance. In a 12,145 procedure study, the revision rate was 1.39% and the mean interval to revision was 3.5 years; younger age was associated with higher risk (Sagheer et al., 2022). A prospective coblation series of 1,257 children reported 2.6% revision, with particularly increased risk in children younger than two years, those with severe OSA, and those with substantial comorbidity (Amin et al., 2021). A 4,111 patient tertiary center series reported 3.3% revision overall and demonstrated a striking decline in revision rates as institutional experience accumulated, underscoring the importance of technique and learning curve (Cameron et al., 2024).

Long-term efficacy should not be judged from regrowth alone. Visible tonsillar tissue after intracapsular surgery is expected; it becomes clinically relevant only when it produces recurrent obstruction or infection. Conversely, the absence of tonsillar regrowth after extracapsular surgery does not guarantee resolution of pediatric OSA, which is frequently multilevel and may persist in children with obesity, craniofacial abnormalities, Down syndrome, neuromuscular disorders, severe baseline disease, or other upper-airway risk factors. The American Thoracic Society guideline on persistent post adenotonsillectomy OSA reinforces the importance of separating tonsillar surgical failure from persistent OSA caused by non-tonsillar disease (Ehsan et al., 2024).

The 5 year randomized comparison of adenotonsillotomy and adenotonsillectomy is particularly informative. Among children available for long term assessment, obstructive apnea hypopnea index (OAHI) fell from approximately 12 events/hour to well below 1 event/hour in both groups, and OSA-18 scores improved substantially without a significant between-group difference. However, 6 of 39 children initially allocated to adenotonsillotomy subsequently underwent complete tonsillectomy, and attrition was substantial (Sjölander, Borgström, Nerfeldt, & Friberg, 2022). Thus, comparable outcomes among long term responders and a higher probability of later completion surgery can coexist; one does not invalidate the other.

Recent data also challenge the assumption that severe OSA necessarily mandates complete tonsillectomy. A 2025 cohort of children with severe and very severe OSA found no statistically significant postoperative PSG difference between intracapsular and total tonsillectomy; among children with severe OSA, mean postoperative OAHI was 7.5 after intracapsular versus 6.6 after total surgery, while the corresponding values in the very severe subgroup were 7.2 and 8.8 events/hour (Hurly et al., 2025). A 2026 systematic review of severe pediatric OSA found a weighted reduction in AHI/OAHI from approximately 19.6 to 3.0 events/hour after intracapsular/partial surgery (Torretta, De Lucia, Cozzi, De Mari, & Battilocchi, 2026). These studies are encouraging but do not establish universal equivalence, because severe OSA data remain heterogeneous and substantially observational.

For recurrent tonsillitis, the balance is less settled. Intracapsular surgery can reduce the frequency of infections, and selected studies have shown acceptable medium and long term outcomes; nevertheless, residual lymphoid tissue remains susceptible to inflammation, and the evidence base is less mature than for obstructive indications (Çetin & Düzenli, 2020; Lao et al., 2025). In a 2020 pediatric series of 393 children with recurrent tonsillitis, postoperative recurrent tonsillitis occurred in 14 of 174 children treated by tonsillotomy, illustrating that partial surgery can be effective but does not eliminate the biological substrate for future infection (Çetin & Düzenli, 2020). The 2025 meta-analysis likewise found higher pooled odds of postoperative infection and reoperation after tonsillotomy (Lao et al., 2025). For a child whose dominant indication is severe recurrent bacterial tonsillitis, recurrent peritonsillar abscess, or a need for complete

histopathologic assessment, extracapsular surgery therefore retains a compelling rationale.

From a practical pediatric otolaryngology perspective, intracapsular and extracapsular surgery should not be framed as competing procedures from which one must eventually eliminate the other. They solve somewhat different clinical problems. Intracapsular surgery prioritizes low acute morbidity while accepting a small long term probability of residual tissue events; extracapsular surgery prioritizes tissue eradication while accepting greater postoperative tissue injury. Current evidence supports an indication driven, child specific, and preference sensitive choice rather than a universal operative rule. Contemporary expert reviews increasingly place shared decision making at the center of that choice (Li, Heslop, & Soaper, 2026).

2. Terminology and Surgical Concepts

Tonsil surgery is among the most familiar procedures in pediatric otolaryngology, yet its terminology has become increasingly imprecise as tissue-preserving techniques have diversified. This is not merely an editorial problem. The terms tonsillotomy, partial tonsillectomy, subtotal tonsillectomy, intracapsular tonsillectomy, and intracapsular tonsil reduction may refer to operations that leave very different amounts of residual lymphoid tissue. Unless operative depth is described, two studies using the same label may not be evaluating the same intervention. Contemporary reviews identify this definitional variability as an important source of heterogeneity in both efficacy and revision data (Lao et al., 2025).

The 2019 American Academy of Otolaryngology Head and Neck Surgery Foundation (AAO-HNSF) guideline defines tonsillectomy as complete removal of the tonsil, including its capsule, by dissection of the peritonsillar plane. That definition corresponds to what is now commonly called total or extracapsular tonsillectomy. The guideline did not issue recommendations specifically endorsing or rejecting partial intracapsular surgery, reflecting the evidence landscape available during guideline development, when long-term comparative data were far less mature than they are today (Mitchell et al., 2019).

2.1. Extracapsular Tonsillectomy

Extracapsular tonsillectomy therefore refers to complete removal of the palatine tonsil together with the tonsillar capsule. The dissection plane lies lateral to the capsule and exposes the tonsillar fossa. The superior constrictor and associated pharyngeal tissues are not intentionally resected, but the operation removes the lymphoid-tissue/capsule layer that previously covered the surgical bed. The potential advantages are complete removal of tonsillar lymphoid tissue, negligible risk of true tonsillar regrowth, and an intuitively definitive approach for recurrent infection or pathology requiring intact tissue assessment. Its principal disadvantage is the deeper wound produced in the tonsillar fossa (Li et al., 2026).

2.2. Intracapsular tonsillectomy

Intracapsular Tonsillectomy removes most of the tonsillar parenchyma while deliberately preserving the fibrous capsule and a thin layer of lymphoid tissue immediately medial to it. In a near total intracapsular operation, the tonsil is reduced very close to the capsule. This differs from a more conservative tonsillotomy, in which only the obstructing medial portion of the tonsil may be removed, often to the level of the faucial pillars. In practice, however, the literature frequently uses these terms interchangeably (Li et al., 2026; Zahalka et al., 2026).

The distinction matters because residual volume is plausibly linked to both sides of the risk equation. A thicker tissue remnant may further insulate the pharyngeal musculature and vessels, but leaves more lymphoid substrate capable of hypertrophy or infection. A near-capsular intracapsular resection may behave more like a total tonsillectomy in long term effectiveness while preserving some of the acute morbidity advantage. Current studies rarely quantify residual tissue volume systematically, which limits causal interpretation of regrowth and device comparisons (Lao et al., 2025; Li et al., 2026; Zahalka et al., 2026).

The biological basis for tissue preservation is straightforward. The palatine tonsils form part of Waldeyer's ring and consist of mucosa-associated lymphoid tissue involved in antigen sampling and adaptive mucosal immune responses (Arambula, Brown, & Neff, 2021). More importantly from a surgical perspective, retaining the capsule and a small medial tissue layer leaves a biological covering over the deeper tonsillar bed. The clinically observed reductions in pain and hemorrhage after intracapsular surgery are consistent with this anatomical difference (Arambula et al., 2021; Sedgwick, Saunders, & Bateman, 2023).

Evidence in this field has evolved substantially since 2019. The 2020 Cochrane review concluded that tonsillotomy probably produced faster return to normal activity approximately 3.8 days earlier in the analyzable trials but judged longer term evidence for recurrence and reoperation to be of low or very low certainty (Blackshaw et al., 2020). Subsequent prospective series, large database studies, meta-analyses, and longer-term follow up have made the acute morbidity advantage considerably more convincing while clarifying, rather than eliminating, concern about revision surgery (Amin et al., 2021; Virkkunen, Nokso-Koivisto, & Sakki, 2025).

That evolution is clinically important. Earlier literature often posed the question as “Is partial tonsil surgery as effective as total tonsillectomy?” Contemporary evidence permits a more useful formulation: for a defined indication, what additional short term morbidity is avoided by preserving the capsule, and what additional long-term probability of recurrence or revision is accepted in exchange? The answer differs between obstructive disease and recurrent infection, between a two-year-old and a ten year old, and between a routine child and one with severe multilevel OSA.

A further methodological issue is the difference between instrument and extent of resection. Coblation does not necessarily mean intracapsular surgery; coblation can be used to perform extracapsular tonsillectomy. Electrocautery likewise can be used for total dissection or for intracapsular tissue reduction. The microdebrider is most commonly associated with powered intracapsular tonsillectomy, but even microdebrider techniques vary in depth. Radiofrequency (RF) can describe conventional RF tonsillotomy, while coblation itself uses radiofrequency energy to create a plasma field in saline. Studies should therefore be interpreted along two independent axes: where the surgeon operates relative to the capsule, and what instrument is used to remove tissue (Lin, Hajarizadeh, Wood, Selvarajah, & Ahmadi, 2024; Sedgwick et al., 2023).

2.3. Surgical Techniques and Technologies

The operative objective should be defined before selecting the device. In a child with hypertrophic tonsils and OSA, the immediate mechanical objective is reduction of obstructing lymphoid volume. In a child undergoing surgery primarily because of repeated tonsillar infection, the objective may instead include maximal elimination of chronically inflamed lymphoid tissue. Those differing goals explain why the same device may be appropriate for both intracapsular and extracapsular surgery but used in fundamentally different planes.

2.3.1. Microdebrider assisted intracapsular tonsillectomy.

The microdebrider removes tissue mechanically using an oscillating or rotating blade with simultaneous suction. In powered intracapsular tonsillectomy, the tonsil is progressively debulked from its medial surface until the surgeon approaches the fibrous capsule. The anterior pillar can be retracted to expose tissue concealed laterally, allowing a near total reduction while avoiding violation of the capsule. Hemostasis is usually obtained separately, commonly with suction monopolar or bipolar cautery (Zahalka et al., 2026).

The microdebrider has several conceptual advantages. Tissue removal is visually controllable, the capsule can often be recognized as the dissection approaches the lateral boundary, and the surgeon is not dependent on thermal tissue ablation for the entire resection. The disadvantage is that the device itself is not primarily a hemostatic instrument, so separate cautery is usually required. Furthermore, an inexperienced operator can remove too little tissue medially, increasing residual volume, or inadvertently breach the capsule laterally. Technique therefore matters at least as much as the nominal device (Zahalka et al., 2026).

2.3.2. Coblation intracapsular tonsillectomy

Coblation has become one of the most extensively studied intracapsular techniques. A bipolar radiofrequency field is generated in a conductive saline environment, creating an ionized plasma layer that disrupts tissue while permitting simultaneous ablation and hemostasis. The surgeon progressively reduces the tonsil toward the capsule, usually leaving a thin protective layer of

tissue. Contemporary meta analyses and large series support favorable postoperative pain and hemorrhage profiles with this technique (Lin et al., 2024; Zahalka et al., 2026).

The 2021 meta-analysis comparing intracapsular and extracapsular coblation is helpful because it partly separates surgical plane from instrument: both groups underwent coblation, but one preserved the capsule. Late postoperative pain favored intracapsular surgery, with a standardized mean difference of approximately −0.78, whereas differences during the first 48 hours were less clear (Daskalakis et al., 2021). This finding supports the view that the morbidity advantage is not merely a property of coblation; preservation of the capsule itself contributes materially to postoperative recovery (Lin et al., 2024).

The 2024 systematic review specifically evaluating coblation intracapsular tonsillectomy also concluded that postoperative hemorrhage was low and that long-term outcomes were encouraging, although the authors highlighted limitations in long-term evidence. Such analyses should be interpreted alongside potential heterogeneity in surgeon technique and tissue-removal depth. A coblation intracapsular tonsillectomy can range from modest tonsillotomy to almost complete removal of the tonsillar parenchyma (Lin et al., 2024).

2.3.3. Conventional radiofrequency tonsillotomy

Radiofrequency tonsillotomy predates widespread coblation and typically uses thermal or RF energy to reduce tonsillar volume without extracapsular dissection. The historical literature demonstrated that RF tonsillotomy could reduce postoperative morbidity, and RF procedures contributed substantially to the development of the modern tissue preserving concept. More recent comparative literature tends to group RF techniques with other tonsillotomy methods rather than identify a uniquely superior RF outcome. The practical distinction from coblation is important: although both employ radiofrequency energy, conventional RF tissue reduction and plasma mediated coblation are not interchangeable technologies. Contemporary evidence is insufficient to declare conventional RF superior to microdebrider or coblation for pediatric intracapsular surgery (Lao et al., 2025; Torretta et al., 2026).

2.3.4. Electrocautery and diathermy

Monopolar and bipolar electrosurgery remain widely used in extracapsular tonsillectomy, either for dissection, hemostasis, or both. They can also be adapted to intracapsular reduction. The clinical implication is worth emphasizing: electrocautery is not synonymous with total tonsillectomy. A 2025 report described a monopolar intracapsular technique as a potentially cost-effective option, demonstrating that intracapsular surgery need not depend on a premium proprietary device (Arch, Rangarajan, Jatana, & Tobin, 2025).

In the 2024 pediatric comparison by Asulin and colleagues, tonsillotomy was performed with mono- or bipolar diathermy, while the historical tonsillectomy cohort underwent cold steel surgery. Tonsillotomy was associated with lower postoperative bleeding and fewer returns to the operating room. The study is

clinically informative but not a pure comparison of resection depth because the groups came from different treatment eras and used different operative technologies. That limitation illustrates a recurrent problem in this literature: when technique, calendar period, perioperative protocols, and surgeon experience all change together, causation cannot be assigned solely to intracapsular versus extracapsular (Asulin et al., 2024).

2.3.5. Extracapsular dissection

Total tonsillectomy can be performed with cold steel dissection, monopolar cautery, bipolar cautery, coblation, or other instruments. The defining feature is not the device but removal of the capsule from the tonsillar fossa. Extracapsular surgery remains particularly logical when complete removal of tonsillar tissue is part of the therapeutic objective for example, dominant recurrent infection, recurrent peritonsillar abscess, or a lesion requiring complete tissue assessment (Mitchell et al., 2019).

2.4. Perioperative Management

2.4.1. Analgesia

Operative technique does not replace evidence-based perioperative analgesia. The AAO-HNSF guideline recommends a single intraoperative dose of intravenous dexamethasone and postoperative ibuprofen and/or acetaminophen and strongly advises against codeine in children younger than 12 years after tonsillectomy (Mitchell et al., 2019). In children with OSA particularly severe OSA an opioid sparing strategy is especially attractive because postoperative respiratory vulnerability and analgesic requirements coexist. In the 2025 severe OSA study by Hurly et al., postoperative management used acetaminophen and ibuprofen and generally avoided opioids (Hurly et al., 2025).

The surgical approach affects the amount and duration of analgesia needed. The 2023 plasma-ablation meta-analysis found discontinuation of analgesia approximately 4.1 days earlier after intracapsular surgery (Sedgwick et al., 2023). The 2025 meta-analysis reported a mean reduction of approximately 3.19 days in analgesic use with tonsillotomy (Lao et al., 2025). A prospective series of 1,257 coblation intracapsular cases reported a median analgesic requirement of approximately six days (Amin et al., 2021). Thus, intracapsular surgery reduces rather than eliminates postoperative pain; parents should not be counseled that the operation is painless.

The magnitude of the recovery advantage from one contemporary meta-analysis can be visualized as follows

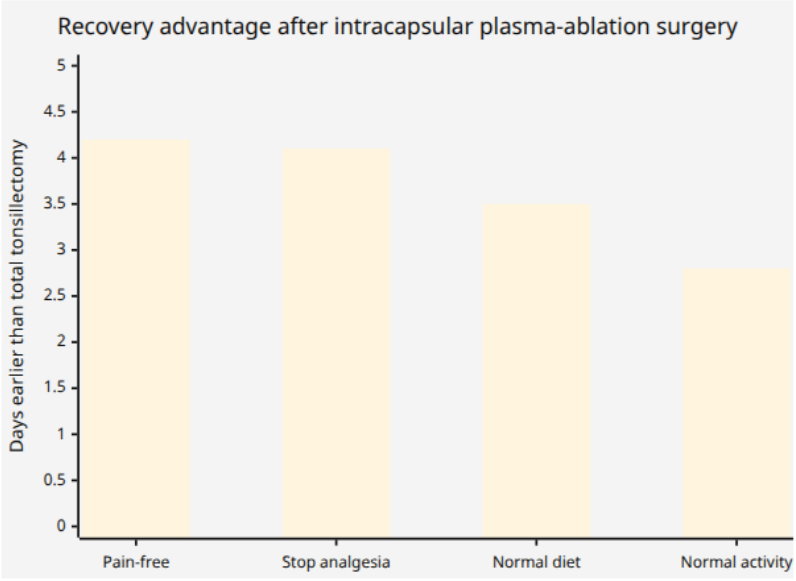


Figure. Synthesized short-term recovery outcomes. Values are pooled mean differences reported by Sedgwick et al. (Sedgwick et al., 2023): pain free 4.2 days earlier, cessation of analgesia 4.1 days earlier, normal diet 3.5 days earlier, and normal activity 2.8 days earlier after intracapsular plasma ablation surgery. These are pooled comparative estimates, not a new meta analysis performed for this chapter.

A clinically important implication is that a three or four day difference is not trivial in a preschool child. Those days may represent the period when oral intake is poorest, caregivers are most anxious about hydration, and families are most likely to seek unplanned medical assessment. Acute recovery therefore deserves the same clinical seriousness as long-term recurrence, even though the two outcomes occur on very different time scales (Lao et al., 2025).

2.4.2. Antibiotics and routine postoperative care

Contemporary pediatric tonsillectomy practice does not support routine perioperative antibiotics solely to improve postoperative recovery in uncomplicated cases; analgesia, hydration, antiemetic management, and bleeding education are more central (Mitchell et al., 2019). Intracapsular surgery does not remove the need for clear discharge instructions concerning oral intake, escalating pain, fever, respiratory symptoms, and any postoperative bleeding. The lower probability of hemorrhage should never be presented as absence of hemorrhage risk.

2.4.3. Postoperative monitoring

Children with young age or severe OSA may still require overnight observation irrespective of intracapsular technique. The surgical plane does not

neutralize OSA related respiratory risk. In severe disease, perioperative planning should be driven by PSG severity, age, comorbidities, airway phenotype, and institutional protocols in addition to the choice of tonsil technique (Ehsan et al., 2024).

3. Indications, Patient Selection, and Clinical Outcomes

The indication for surgery should be considered before the instrument. This principle is central to rational use of intracapsular surgery because the biological consequences of leaving residual tissue differ according to whether the child is being treated for mechanical obstruction or recurrent infection.

3.1. Obstructive sleep-disordered breathing and OSA.

The most compelling indication for intracapsular surgery is adenotonsillar hypertrophy causing oSDB or OSA. In this setting, the therapeutic objective is airway enlargement. There is no inherent physiological requirement to remove every microscopic focus of tonsillar lymphoid tissue if adequate pharyngeal patency can be achieved by reducing tonsil bulk. This makes capsule-preserving surgery conceptually well matched to the disease (Blackshaw et al., 2020; Lao et al., 2025).

Clinical outcomes must nevertheless be separated into several domains: snoring and respiratory symptoms, disease specific quality of life, PSG parameters, residual OSA, regrowth, and revision surgery. An operation can perform well in one domain and less well in another. For example, a child may experience durable symptomatic relief despite visible residual tonsil tissue, while another may have technically complete extracapsular surgery but persistent OSA because obesity, tongue base obstruction, craniofacial anatomy, or other levels of airway collapse remain untreated (Ehsan et al., 2024).

3.2. PSG outcomes

PSG outcomes. The 5 year randomized trial by Sjölander and colleagues provides the most clinically persuasive long-term comparative PSG evidence available. Children initially underwent adenotonsillotomy or adenotonsillectomy for OSA. Among those assessed at five years, mean OAHI fell from approximately 12.3 to 0.6 events/hour after adenotonsillectomy and from approximately 12.6 to 0.5 after adenotonsillotomy, with no clinically meaningful postoperative between group difference. These findings support the view that appropriately performed partial surgery can provide durable airway improvement in selected otherwise healthy children (Sjölander et al., 2022).

The same trial also demonstrates why outcome interpretation requires restraint. Only 45 of the original 79 children completed the five-year assessment, and six children initially assigned to adenotonsillotomy subsequently underwent complete tonsillectomy. An analysis restricted to children remaining in their original groups can therefore demonstrate excellent long-term effectiveness among responders while underrepresenting the clinical relevance of completion surgery. For counseling purposes, both observations matter: the respiratory result

can be excellent, but the probability of a second operation is not zero (Sjölander et al., 2022).

The 2024 prospective study by Lildal et al. similarly documented improvement after tonsillotomy in children with oSDB, including children with moderate-to severe disease. The exact effect estimates vary by analysis and clinical subgroup; where numerical values were not consistently available in the accessible primary abstract, they are intentionally left unspecified here rather than reconstructed from secondary citations. The importance of the study lies in its prospective demonstration that partial surgery can produce meaningful clinical and sleep-related improvement beyond mildly symptomatic children (Lildal et al., 2024).

3.3. Severe OSA

One of the more consequential developments in the recent literature is the expansion of intracapsular surgery into children with severe OSA. Historically, some surgeons favored complete tonsillectomy in severe disease on the premise that maximal tissue removal should yield maximal airway benefit. Recent PSG data do not support that assumption as a universal rule. Hurly et al. studied 57 children with severe OSA, defined as OAHI ≥10 events/hour, including a subgroup with very severe disease defined as OAHI ≥30. In the full severe cohort, preoperative mean OAHI was 45.9 in the total group and 36.3 in the intracapsular group; postoperative OAHI was 6.6 and 7.5, respectively, without a significant between-group difference. Among the very severe subgroup, postoperative OAHI was 8.8 after total surgery and 7.2 after intracapsular surgery, again without a significant difference. The study was small and retrospective, but it directly challenges the proposition that intracapsular surgery is inherently inadequate in severe disease (Hurly et al., 2025).

The 2026 systematic review focused specifically on severe pediatric OSA provides broader support. Across analyzable studies, weighted mean AHI/OAHI decreased from approximately 19.6 events/hour preoperatively to 3.0 postoperatively, corresponding to a weighted reduction of about 16.5 events/hour. The authors nevertheless emphasized heterogeneity, the absence of demonstrated superiority of a particular instrument, and the need for surveillance for residual OSA and regrowth. Severe OSA can therefore be considered an eligible indication for intracapsular surgery in experienced hands, but not an indication in which postoperative reassessment should be relaxed (Torretta et al., 2026).

3.4. OSA-18 and quality of life

Polysomnography and symptom burden answer different questions. OSA-18 is a parent reported disease specific quality of life instrument that captures sleep disturbance, physical symptoms, emotional distress, daytime function, and caregiver concerns. The five year randomized study found marked improvement in OSA-18 in both surgical groups: median scores fell from approximately 57 to 27 after adenotonsillectomy and from 67 to 32 after adenotonsillotomy, without a significant long-term between group difference.

The 2025 systematic review and meta-analysis similarly found no significant difference between tonsillotomy and tonsillectomy in postoperative OSA-18 improvement (pooled mean difference 5.20, 95% CI −32.67 to 43.07; p=0.96). [20] The broad confidence interval indicates heterogeneity and limited precision, but the direction of the totality of evidence does not suggest a large symptomatic disadvantage from intracapsular surgery in children treated primarily for oSDB (Sjölander et al., 2022).

Persistent OSA should not automatically be called regrowth. This distinction is especially important in obese children and in children with Down syndrome, craniofacial disorders, neuromuscular disease, lingual tonsillar hypertrophy, or other sites of airway obstruction. Persistent postoperative disease may occur despite technically complete extracapsular tonsillectomy. The 2024 ATS clinical practice guideline treats persistent postadenotonsillectomy OSA as a separate therapeutic entity and considers interventions directed at the child's remaining anatomical and physiological contributors.

Accordingly, a child who remains symptomatic after intracapsular surgery should not proceed automatically to completion tonsillectomy without confirming the source of obstruction. Examination for true tonsillar regrowth, assessment of adenoid tissue, nasal obstruction, obesity, craniofacial structure, tongue base, and when appropriate repeat PSG or dynamic airway assessment may be more informative than assuming the residual tonsil is responsible (Ehsan et al., 2024).

3.5. Recurrent tonsillitis

The rationale becomes more complex when infection is the dominant indication. Here, residual tonsillar tissue is not merely an anatomical remnant; it remains immunologically active lymphoid tissue capable of recurrent inflammation. The 2020 study by Çetin and Düzenli compared bipolar tonsillotomy with total tonsillectomy in 393 children treated for recurrent tonsillitis. Fourteen of 174 children in the tonsillotomy group experienced postoperative recurrent tonsillitis, and overall outcomes were sufficiently favorable for the authors to consider the procedure an effective alternative in selected patients (Çetin & Düzenli, 2020).

Longer term data are somewhat reassuring. The 12 year follow-up published by Virkkunen, Nokso-Koivisto, and Sakki found no evidence that tonsillotomy produced a clearly greater long-term burden of tonsillar infection than tonsillectomy in the followed cohort. Such findings argue against the simplistic assertion that residual tissue inevitably leads to clinically important recurrent infection. However, they do not establish that intracapsular surgery should replace total tonsillectomy for every child meeting recurrent-infection criteria (Virkkunen et al., 2025).

The larger 2025 meta analysis offers an important counterpoint: compared with total tonsillectomy, tonsillotomy was associated with increased pooled odds of postoperative infection (OR 1.82, 95% CI 1.34–2.47), oSDB recurrence (OR

2.16, 95% CI 1.20–3.86), and reoperation (reported pooled OR 8.28). These relative measures should not be confused with absolute probabilities; when baseline revision rates are low, a high relative odds ratio can still correspond to a modest absolute event rate. Nonetheless, the direction of effect is clinically relevant and should be part of informed consent (Lao et al., 2025).

For that reason, extracapsular surgery remains particularly reasonable when recurrent infection is severe, frequent, or the dominant disease burden; when recurrent peritonsillar abscess has occurred; or when complete tissue removal is desired for diagnostic reasons. Intracapsular surgery may remain reasonable in a child with mixed obstruction and infection when obstruction is clearly dominant and the family places high value on reducing postoperative morbidity. Recent expert review supports this individualized approach rather than an absolute prohibition of intracapsular surgery for infection (Li et al., 2026).

Age as a selection variable. Young children create one of the most interesting trade offs. They stand to gain substantially from less painful surgery because maintaining oral intake is more difficult and caregiver concern about hydration is greater. Yet several large datasets show that younger age also increases the probability of eventual revision. In the Amin series, children younger than two years had an odds ratio of 5.10 for revision (Amin et al., 2021). Sagheer et al. identified age younger than four years as a revision risk factor (Sagheer et al., 2022). Cameron et al. also found significantly greater revision risk in children younger than two years (Cameron et al., 2024).

This does not make young age a contraindication. It makes age a counseling variable. For a two year-old with severe obstructive hypertrophy, a family may reasonably prefer the lower acute morbidity of intracapsular surgery despite accepting a higher lifetime opportunity for regrowth. Another family may prefer a more definitive extracapsular procedure specifically to minimize the likelihood of a second anesthetic. Both choices can be defensible.

3.6. Comorbidity and baseline severity

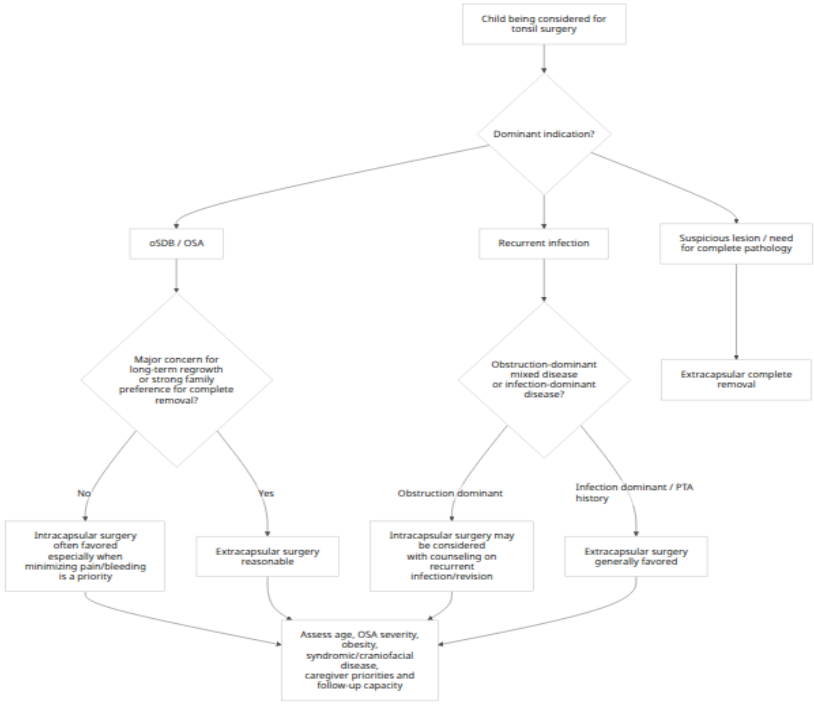
In the 1,257 child prospective coblation series, severe OSA and severe comorbidities were independently associated with revision, with reported odds ratios of 4.43 and 2.98, respectively (Amin et al., 2021). These data suggest that complex disease should modify follow-up expectations rather than automatically exclude intracapsular surgery. Conversely, in the 2026 microdebrider versus coblation cohort, age and baseline tonsil size did not independently predict revision after adjustment, illustrating that predictors are not uniform across institutions or techniques (Zahalka et al., 2026).

3.7. Suspicious or asymmetric tonsillar pathology

When there is meaningful concern for malignancy or another condition requiring complete specimen evaluation, tissue reduction is conceptually inappropriate. Complete removal permits definitive pathological assessment and avoids leaving potentially abnormal tissue behind. This remains a straightforward

scenario in which the advantages of intracapsular surgery are subordinate to diagnostic completeness (Li et al., 2026).

A practical clinical pathway can therefore be framed as follows:



This framework is a synthesis of the contemporary evidence rather than a formal guideline algorithm

3.8. Hemorrhage, Recovery, Regrowth, and Revision

Postoperative morbidity is where the evidence favoring intracapsular surgery is strongest. The advantage is reproducible across systematic reviews, large retrospective cohorts, and prospective series, although the exact event rates differ substantially because studies define bleeding differently and use different instruments, patient populations, and follow-up systems.

3.8.1. Postoperative hemorrhage

The 2023 plasma ablation systematic review included 17 studies encompassing 1,996 intracapsular procedures and 4,565 total tonsillectomies. Overall postoperative hemorrhage was significantly less common after intracapsular surgery, with RR 0.36 (95% CI 0.16–0.81). The estimate for bleeding requiring operative intervention also favored intracapsular surgery but was less precise and did not demonstrate the same statistical certainty (Sedgwick et al., 2023).

The 2025 meta analysis of 32 studies and 9,430 children reported a similar reduction in secondary postoperative bleeding with tonsillotomy: RR 0.33 (95% CI 0.23–0.47). [20] The agreement between these two meta analyses is clinically persuasive because they span different study sets and definitions yet arrive at a very similar relative reduction (Lao et al., 2025).

Large real world cohorts reinforce the pooled findings. In the 2024 Australasian study of 12,275 procedures, postoperative hemorrhage occurred in 1.7% of intracapsular procedures versus 4.1% after extracapsular surgery. Return to the operating room for hemorrhage occurred in approximately 0.08% versus 0.93%, respectively. These are clinically meaningful absolute differences, particularly when the operation is performed in young children (Loh et al., 2024).

Asulin et al. reported a similar direction of effect in 1,984 pediatric patients: bleeding was documented in 3.9% after tonsillotomy versus 9.5% after tonsillectomy, while operative control of bleeding was required in 0.5% versus 3.7%. Interpretation requires caution because the total tonsillectomy cohort and tonsillotomy cohort were drawn from different historical periods and employed different techniques. The magnitude should therefore not be treated as a universal causal estimate, but the findings are consistent with the broader literatüre (Asulin et al., 2024).

The 2023 US national cohort by Johnson and colleagues provides context for conventional pediatric tonsillectomy. In more than 96,000 children, approximately 2.2% had a return encounter for postoperative bleeding, underscoring that hemorrhage remains an important quality metric even outside technique comparison studies (Johnson et al., 2023).

Bleeding definitions deserve explicit attention. Any blood in saliva, a telephone report of bleeding, emergency department evaluation, hospital readmission, and return to theatre are not interchangeable endpoints. Studies employing active diaries will inevitably record more minor events than database studies capturing only hospital encounters. The most clinically robust comparative outcomes are therefore severe bleeding requiring healthcare contact, admission, transfusion, or operative intervention, not simply any postoperative blood staining.

3.8.2. Pain and return to function

The recovery advantage is similarly consistent. The 2023 meta-analysis showed children undergoing intracapsular plasma-ablation surgery became pain free about 4.2 days earlier, stopped analgesics 4.1 days earlier, resumed normal diet 3.5 days earlier, and resumed normal activity 2.8 days earlier (Sedgwick et al., 2023). The 2025 meta-analysis reported normal diet approximately 2.49 days earlier and cessation of analgesics approximately 3.19 days earlier after tonsillotomy (Lao et al., 2025).

These outcomes are especially relevant because post tonsil surgery morbidity is cumulative. Pain causes reduced swallowing; reduced swallowing can reduce fluid intake; dehydration worsens fatigue and nausea; poor oral intake can drive

emergency consultation. A technically uncomplicated operation can therefore generate substantial family burden without a major complication. The intracapsular recovery advantage should not be dismissed as merely a comfort outcome.

3.8.3. The regrowth

The regrowth problem must be defined correctly. Residual tonsil tissue is an intended feature of intracapsular surgery. Seeing residual lymphoid tissue months or years later is not, by itself, a complication. Three distinct outcomes should be separated: anatomical regrowth means an increase in visible residual tissue; symptomatic recurrence means return of clinically meaningful obstruction or infection; and revision surgery means symptoms have become sufficiently important to justify another operation.

Studies that report one of these outcomes should not be used as though they report all three. Revision surgery is the most objective and clinically meaningful of the three, but it is strongly influenced by follow-up duration and local practice. A 12-month study will miss revisions occurring at age six after surgery at age two. Indeed, Sagheer et al. found a mean interval of 3.5 years from initial intracapsular tonsillectomy to revision in their large cohort (Sagheer et al., 2022). Short follow-up therefore systematically underestimates the main long-term disadvantage of intracapsular surgery.

3.8.4. Large scale revision evidence

Sagheer and colleagues reviewed 12,145 intracapsular procedures and reported a 1.39% revision rate. Younger age particularly under four years and gastroesophageal reflux disease were associated with increased revision risk. Among children who did require revision, tonsillitis was an important indication, and postoperative hemorrhage requiring operative control after the revision procedure occurred in a small minority. This study is valuable because of its sample size and long interval to revision (Sagheer et al., 2022).

The prospective 1,257 case coblation series by Amin et al. reported a 2.6% revision rate, predominantly for recurrent obstruction. Age younger than two years was associated with a markedly increased revision risk (OR 5.10), as were severe OSA (OR 4.43) and severe comorbidity (OR 2.98) (Amin et al., 2021). The same cohort reported no return to theatre for postoperative hemorrhage in the primary series, while most children resumed normal diet within 24 hours and usual activity or school within approximately one week. The study therefore illustrates the central intracapsular trade-off unusually well: excellent acute safety and recovery, with a small later revision burden.

Cameron et al. reported 135 revisions among 4,111 children treated with coblation intracapsular tonsillectomy, corresponding to 3.3% overall. Perhaps more interesting than the aggregate rate was the temporal pattern: revision frequency fell from approximately 10.6% during the earliest phase of the institutional experience to approximately 0.3% in the latest period. Children younger than two years again had higher revision risk. This dramatic temporal

decline strongly suggests that surgeon experience, procedural standardization, and perhaps progressively more complete intracapsular tissue reduction influence long-term failure (Cameron et al., 2024).

That observation has major implications for interpreting device comparisons. A device introduced late in an institution's experience may appear better because surgeons have already refined their understanding of the correct resection depth. Conversely, a new device may appear worse because its early cases coincide with a learning curve.

The 2026 microdebrider versus coblation study by Zahalka and colleagues illustrates precisely this concern. In 827 children undergoing partial intracapsular tonsillectomy and adenoidectomy for oSDB, 31 revisions occurred: six after microdebrider surgery and 25 after coblation. Thirty six month revision free survival was approximately 99.4% for microdebrider versus 96.1% for coblation, and adjusted analysis strongly favored the microdebrider group (Zahalka et al., 2026). The authors themselves cautioned that the finding could reflect institutional experience, procedural execution, and the period in which coblation was introduced rather than an intrinsic biological inferiority of coblation.

This is a crucial point. It would be premature to conclude from one retrospective cohort that microdebrider is definitively superior to coblation. Contemporary severe OSA reviews likewise have not demonstrated clear superiority of any one intracapsular instrument (Torretta et al., 2026; Zahalka et al., 2026). A device should therefore be selected according to the surgeon's ability to achieve reproducible near capsular reduction with secure hemostasis, not according to marketing claims or one uncontrolled comparison.

3.9. Long-term comparative outcomes

The 2025 12 year study by Virkkunen et al. is important because it addresses a central limitation of earlier literature: inadequate duration of follow-up (Virkkunen et al., 2025). Over a median long-term interval of approximately 12 years, tonsillotomy was not shown to create a clearly excessive burden of later tonsillar infection compared with tonsillectomy. Long follow-up is particularly valuable because children operated on during preschool years pass through the entire period of peak lymphoid growth.

The randomized five year data are somewhat more cautionary. Six of 39 children originally treated with adenotonsillotomy subsequently underwent complete tonsillectomy (Sjölander et al., 2022). This proportion is higher than many large retrospective revision rates, and the reasons include differences in operative technique, patient population, and study protocol. The discrepancy should not be averaged away. It shows that revision frequency is not a fixed property of the word intracapsular; it is a property of a specific operation in a specific child over a specific duration of follow up (Lao et al., 2025).

Age and technique together. The most consistent age signal across the literature is increased revision risk in very young children, particularly below two to four years (Amin et al., 2021; Sagheer et al., 2022). The 2026 device study did

not reproduce age as an independent predictor, illustrating that age effect can be modified by technique, selection, and follow-up (Zahalka et al., 2026). A plausible clinical interpretation is that younger children have more years of lymphoid growth ahead of them and may undergo surgery at a time of active immune tissue expansion, but observational associations should not be converted into a deterministic biological rule.

3.10. Immune implications

The palatine tonsils participate in mucosal immune surveillance and antigen processing, so it is reasonable to ask whether preserving some tissue confers an immunologic benefit (Arambula et al., 2021). This theoretical advantage is often raised in discussions of tonsillotomy, but the evidence should be presented carefully. Tonsillar immune function does not imply that complete tonsillectomy produces clinically meaningful immune deficiency. Contemporary reviews of adenotonsillar surgery have found laboratory immune changes in some studies but have not established a consistent, clinically important long-term immunodeficiency attributable to tonsillectomy (Liu et al., 2024).

Small studies of partial surgery likewise suggest that measured immunoglobulin or inflammatory indices are not substantially impaired after tonsillotomy. A 2023 study in children with chronic tonsillitis reported improvement in inflammatory disease burden without evidence of a major short term adverse effect on the immune measures studied (Wu et al., 2023). Such findings are reassuring but do not prove that intracapsular surgery provides a clinically relevant immüne preservation advantage. The appropriate conclusion is therefore conservative: immune preservation is biologically plausible, but it should not currently be a primary reason to choose intracapsular rather than extracapsular surgery.

4. Comparative Judgment, Controversies, and Shared Decision Making

No contemporary interpretation of this literature is well served by asking which operation is better without specifying the child, indication, time horizon, and outcome. Intracapsular surgery is clearly better for some short-term outcomes; extracapsular surgery is clearly more definitive in eliminating tonsillar tissue. The clinical decision lies in assigning appropriate weight to those different objectives.

Domain	Intracapsular surgery	Extracapsular surgery	Preferred clinical scenarios
Operative principle	Preserves capsule and a variable thin rim of lymphoid tissue	Removes tonsil and capsule in the peritonsillar plane	Determined by therapeutic objective rather than device
Postoperative pain	Consistently lower; earlier pain resolution and shorter analgesic requirement	Greater and more prolonged tonsillar-fossa pain	Intracapsular favored when minimizing recovery burden is a priority
Postoperative hemorrhage	Lower pooled and large-cohort bleeding rates	Higher bleeding risk, particularly secondary hemorrhage	Intracapsular particularly attractive in young children and when bleeding avoidance is highly valued
Diet/activity recovery	Earlier return to normal diet and activity	Slower recovery on average	Intracapsular favored for outpatient recovery and reduced caregiver burden
OSA/oSDB efficacy	Strong evidence of symptomatic and PSG improvement; comparable outcomes in several comparative studies	Effective and definitive tonsillar-volume removal	Intracapsular often favored for adenotonsillar hypertrophy/oSDB; either appropriate with individualized counseling
Severe OSA	Emerging evidence supports substantial PSG improvement; long-term evidence still developing	Historically preferred by some surgeons for maximal tissue removal	Technique alone should not dictate choice; postoperative monitoring and persistent-OSA assessment remain essential
Recurrent tonsillitis	Can reduce infection burden, but residual tissue remains susceptible to future infection	Maximally eliminates palatine tonsillar tissue	Extracapsular generally favored when recurrent infection is the dominant indication; intracapsular reasonable in selected mixed cases
Regrowth/revision	Small but real long-term risk;	True tonsillar regrowth is	Extracapsular favored when

Domain	Intracapsular surgery	Extracapsular surgery	Preferred clinical scenarios
	higher in some younger children and technique-dependent series	essentially avoided	family prioritizes minimizing future completion surgery
Histopathologic need	Leaves tissue behind and may fragment specimen	Provides complete tonsil for assessment	Extracapsular favored when malignancy or significant unilateral pathology is suspected
Immune preservation	Retains viable tonsillar lymphoid tissue, but clinical immune advantage remains unproven	Removes palatine tonsil; clinically important long-term immune deficiency has not been established	Immune considerations alone should not determine technique
Device flexibility	Microdebrider, coblation, RF, monopolar/bipolar techniques possible	Cold steel, monopolar/bipolar cautery, coblation and other devices possible	Surgeon reproducibility and correct surgical plane matter more than device branding
Core limitation	Possibility of regrowth, recurrent infection, and revision	Greater acute pain, tissue injury, and hemorrhage risk	Shared decision should explicitly compare acute morbidity with long-term definitiveness

Table. Intracapsular vs Extracapsular: advantages, limitations, preferred scenarios. Synthesis based primarily on contemporary systematic reviews, large cohorts, long-term follow-up, and guidelines

Is intracapsular surgery becoming the preferred operation for pediatric OSA? In a straightforward child with hypertrophic tonsils and oSDB, the argument in favor is increasingly strong. The short-term advantages are consistent and clinically meaningful, while PSG and quality of life outcomes are generally comparable in the available trials and cohorts. For many pediatric otolaryngologists, the critical question has shifted from whether intracapsular surgery works to whether a specific child has a reason to prefer complete extracapsular removal.

Calling intracapsular surgery the universal new standard would nevertheless go beyond the evidence. Definitions remain heterogeneous; high-quality randomized long term data are sparse; complex syndromic children are underrepresented in many trials; revision rates vary between institutions; and the

2019 AAO-HNSF guideline was not constructed around an intracapsular versus extracapsular recommendation (Blackshaw et al., 2020; Mitchell et al., 2019). The appropriate current statement is that intracapsular surgery is an established, evidence supported option and often an attractive one for pediatric obstructive disease, not that extracapsular tonsillectomy has become obsolete.

How much tissue should remain? This may be more important than which device is used. A superficial tonsillotomy and a near total intracapsular tonsillectomy are both partial operations but leave very different volumes of tissue. Existing studies rarely document residual volume quantitatively. Until operative depth is standardized, meta-analyses will continue to pool procedures that are biologically non-equivalent (Lao et al., 2025; Zahalka et al., 2026).

A practical surgical objective is to preserve the capsule rather than preserve a large amount of tonsil. This distinction is important. The intended protective structure is the intact lateral tissue/capsule interface; leaving substantial medial bulk is not necessarily required to obtain the morbidity advantage and may increase the substrate for later obstruction. This interpretation is consistent with the dramatic improvement in revision rates seen as some institutional programs gained experience, although causation has not been conclusively demonstrated (Cameron et al., 2024; Li et al., 2026).

Does severe OSA require total tonsillectomy? Current evidence does not support severity alone as an absolute reason to remove the capsule. Hurly et al. found comparable postoperative OAHI outcomes in severe and very severe OSA, and the 2026 systematic review demonstrated a substantial pooled decrease in AHI/OAHI after intracapsular procedures. What severe OSA does require is appropriate perioperative monitoring and an understanding that residual disease may persist because OSA is multilevel and multifactorial. This distinction changes counseling. Families should not be told that intracapsular surgery necessarily compromises OSA treatment because the baseline AHI is high. They should instead be told that severe OSA makes postoperative evaluation more important and that neither total nor intracapsular tonsil surgery can guarantee normalization of PSG when other airway risk factors are present (Hurly et al., 2025; Torretta et al., 2026).

Should intracapsular surgery be used for recurrent tonsillitis? This remains one of the most legitimate areas of disagreement. Moderate and long term observational studies demonstrate that many children improve substantially after tonsillotomy, and the 2026 expert review considers recurrent tonsillitis a potential indication (Çetin & Düzenli, 2020; Li et al., 2026; Virkkunen et al., 2025). Conversely, the 2025 meta-analysis found higher odds of postoperative infection and reoperation after tonsillotomy, which is biologically plausible because residual lymphoid tissue remains (Lao et al., 2025).

In clinical practice, the indication should therefore be characterized more precisely than tonsillitis. A child with large tonsils, nightly obstruction, and two or three infectious episodes may reasonably be treated as an obstruction-dominant patient. A child with minimal obstruction and repeated well-documented bacterial

tonsillitis or peritonsillar abscess has a different disease phenotype and a stronger rationale for complete removal. Studies that combine these phenotypes risk obscuring the very distinction that should drive operative choice.

Is one intracapsular device superior? At present, no. Coblation has the largest contemporary outcome literature and favorable systematic review data (Amin et al., 2021; Daskalakis et al., 2021; Lin et al., 2024). Microdebrider techniques also have extensive historical and contemporary experience and permit controlled mechanical reduction. The 2026 retrospective comparison suggested fewer revisions after microdebrider than coblation within one institution, but the investigators emphasized potential confounding by era and technical experience (Zahalka et al., 2026). Monopolar intracapsular surgery is emerging as a lower cost alternative, while RF tonsillotomy remains a viable tissue reduction concept (Arch et al., 2025). There is insufficient controlled evidence to establish a universal device hierarchy.

The more defensible hierarchy is therefore experienced surgeon + standardized near capsular technique + reliable hemostasis > particular proprietary device.

Does preserving tonsil tissue preserve immunity? Anatomically, yes: living lymphoid tissue remains. Clinically, however, there is no persuasive evidence that this translates into a meaningful reduction in infections, immune disorders, or other patient centered outcomes compared with total tonsillectomy (Liu et al., 2024; Wu et al., 2023). Likewise, current evidence does not demonstrate clinically important chronic immune deficiency after conventional tonsillectomy. Immune preservation is therefore an interesting mechanistic argument but a weak basis for choosing the operation (Li et al., 2026).

Industry and evidence interpretation. The field also deserves the same critical appraisal applied to any device-heavy surgical literature. Some studies evaluate proprietary plasma ablation platforms, and some publications have disclosed author relationships with device manufacturers. For example, the 2023 plasma ablation systematic review disclosed relevant industry relationships among authors (Sedgwick et al., 2023). This does not invalidate the results, particularly because independent cohorts and later meta analyses show the same general direction of effect, but readers should separate a reproducible intracapsular principle from claims of superiority for a specific commercial technology (Lin et al., 2024).

Shared decision making. The decision between intracapsular and extracapsular surgery is unusually well suited to shared decision making because the trade-off involves outcomes that families value differently. There is rarely a single numerical endpoint that determines the choice. A family whose greatest concern is post-tonsillectomy hemorrhage after a difficult previous family experience may strongly prefer intracapsular surgery. A family living far from tertiary care may likewise value the lower acute bleeding risk. Another family may be particularly concerned about the possibility of a second anesthetic and prefer total tonsillectomy despite a more painful recovery (Li et al., 2026).

Counseling is most useful when expressed in absolute clinical terms rather than labels such as safer or more definitive. A balanced discussion might be framed as follows:

For intracapsular surgery: most evidence indicates less pain, less analgesic use, faster eating and activity, and a lower postoperative bleeding risk. The operation is highly effective for obstructive disease, but some tonsil tissue intentionally remains, so a small proportion of children develop recurrent obstruction or infection and eventually require another tonsil operation. Younger children may carry a greater lifetime revision risk (Amin et al., 2021; Lao et al., 2025; Sagheer et al., 2022).

For extracapsular surgery: the tonsil and its capsule are completely removed, so clinically meaningful tonsillar regrowth is essentially avoided and the approach remains particularly logical for infection dominant disease or when complete pathology is required. The price of that definitiveness is a deeper surgical wound, greater postoperative pain on average, slower return to normal oral intake, and a higher bleeding risk (Asulin et al., 2024; Loh et al., 2024).

The discussion becomes particularly nuanced in a very young child. A two year old may benefit disproportionately from reduced pain and lower hemorrhage risk, yet the same age confers more years in which residual lymphoid tissue can enlarge (Amin et al., 2021; Cameron et al., 2024). This is not a contradiction; it is precisely why preference-sensitive counseling is necessary.

The same principle applies to severe OSA. Intracapsular surgery can be effective, but the family should understand that postoperative PSG abnormalities may persist irrespective of technique and that severe baseline disease may warrant closer surveillance (Ehsan et al., 2024; Hurly et al., 2025). In that setting, promising comparative PSG data should be presented alongside their limitations rather than used to promise equivalence in every patient.

Ultimately, the key distinction is between short-term morbidity and long-term definitiveness. Intracapsular surgery moves the balance toward reduced acute morbidity. Extracapsular surgery moves it toward maximal tissue eradication. The modern surgeon's task is not to defend one operation philosophically, but to determine which balance is appropriate for the child in front of them.

5. Future Research Priorities

Despite a rapidly expanding evidence base, the next generation of studies should be designed differently from much of the existing literature. Merely adding another retrospective comparison of intracapsular versus extracapsular tonsillectomy without standardizing what those terms mean is unlikely to resolve the remaining controversies.

Standardized operative nomenclature is the first priority. Future studies should document not only whether surgery was intracapsular but how much tissue was intentionally left behind. A useful operative classification might distinguish limited tonsillotomy, pillar level reduction, near total intracapsular removal, and

true extracapsular dissection. Device, energy settings, method of hemostasis, capsule violation, and surgeon experience should be recorded separately. The pronounced institutional learning-curve effects observed in contemporary revision studies make this particularly important.

Outcome definitions require standardization. Postoperative hemorrhage should be graded by clinical consequence: self reported blood staining, medical contact, emergency department attendance, hospital admission, transfusion, and return to theatre should not be collapsed into one endpoint. Similarly, tonsillar regrowth should distinguish anatomical regrowth from symptomatic recurrence and revision surgery. Without these distinctions reported event rates from different institutions cannot be meaningfully compared.

Long term follow up should be measured in years rather than months. A mean revision interval of approximately 3.5 years in the 12,145 case study demonstrates why one year studies are inadequate for judging durability (Sagheer et al., 2022). Five and ten year outcomes are particularly important in children undergoing surgery before school age. The 12 year follow-up study represents the type of longitudinal evidence the field needs more often (Virkkunen et al., 2025).

Time to event analysis should replace simple revision percentages. A reported revision rate of 2% after two years is not equivalent to 2% after ten years. Kaplan Meier revision free survival and Cox regression are more informative because they incorporate different follow up durations and allow assessment of age, indication, device, residual tonsil volume, obesity, comorbidity, and surgeon experience. The 2026 device study's use of revision free survival illustrates the usefulness of this approach, even though residual confounding remained (Zahalka et al., 2026).

Obstructive and infectious indications should be analyzed separately. Combining an OSA dominant preschool child with an older child undergoing surgery for recurrent streptococcal tonsillitis produces a clinically heterogeneous cohort. The mechanism of failure is different: recurrent obstruction may reflect lymphoid regrowth, while recurrent infection reflects continued susceptibility of residual tissue. Future trials should prespecify indication and ideally analyze mixed disease phenotypes separately. The 2025 meta analysis demonstrates that long term infection and recurrent oSDB do not behave identically (Lao et al., 2025).

PSG and patient-reported outcomes should coexist. OAHI and oxygen saturation quantify physiological disease; OSA-18 and related instruments quantify what families observe and experience. Neither should substitute for the other. The five year randomized trial illustrates the value of demonstrating parallel improvement in PSG and quality of life measures (Sjölander et al., 2022). In complex children and those with severe OSA, postoperative PSG is especially important because residual disease may be clinically silent or attributed incorrectly to tonsillar regrowth (Ehsan et al., 2024; Hurly et al., 2025).

Severe OSA deserves prospective multicenter study. Recent evidence is encouraging, but the available severe OSA literature remains dominated by small or observational cohorts. A pragmatic multicenter trial comparing near total intracapsular and extracapsular surgery in children stratified by baseline OAHI, obesity, Down syndrome, craniofacial diagnosis, and age would be considerably more informative than additional unstratified case series. Such a study should include immediate respiratory events, six to twelve month PSG, quality of life, and five year revision free survival (Hurly et al., 2025).

Device comparisons require control of surgeon experience. The 2026 microdebrider versus coblation findings are hypothesis generating, not definitive (Zahalka et al., 2026). Future head to head studies should randomize or at least prospectively standardize resection depth and ensure surgeons are beyond their learning curves with both technologies. Otherwise, the comparison measures operator familiarity as much as device performance.

Cost-effectiveness should include family costs. Operative device cost is only one component. A more meaningful economic evaluation should include operating room time, unplanned emergency visits, hemorrhage related readmissions, repeat surgery, postoperative medications, parental time away from work, and days missed from school or childcare. A more expensive disposable device could still be cost effective if it meaningfully reduces bleeding and recovery burden; conversely, a low-cost monopolar intracapsular technique may offer much of the same benefit if outcomes are reproducible (Arch et al., 2025).

Immune research should focus on clinical rather than laboratory endpoints. Additional short term measurements of immunoglobulin levels are unlikely to settle the immüne preservation debate. Future studies would be more informative if they examined clinically relevant infection patterns, vaccine responses, immüne mediated disease, or validated long term outcomes while rigorously controlling for confounding. At present, neither presumed immune preservation after intracapsular surgery nor presumed immune harm after total tonsillectomy has sufficient evidence to dominate operative decision making (Arambula et al., 2021; Liu et al., 2024; Wu et al., 2023).

Shared decision making deserves formal study. The literature largely measures what surgeons value bleeding, PSG indices, operative time, and revision but not necessarily how families trade these outcomes against one another. Discrete choice studies could quantify how parents value, for example, a three day reduction in pain versus a 1–3% long-term probability of revision surgery. Such research would directly inform the preference-sensitive decision that contemporary expert reviews already advocate (Li et al., 2026).

The most useful future trial would therefore not simply ask whether intracapsular surgery is non inferior. It would determine, for clearly defined patient phenotypes, the absolute reduction in acute morbidity obtained for each absolute increase in long term revision risk, while documenting PSG, quality of life, infection burden, cost, and family preference over a sufficiently long follow up period.

The clinical evidence available through September 2026 supports a relatively mature conclusion. Intracapsular tonsil surgery is no longer an experimental or marginal alternative to conventional tonsillectomy. For pediatric oSDB, it is an established strategy with a reproducible advantage in postoperative pain, recovery, and hemorrhage and with generally comparable symptom and PSG improvement in appropriately selected children.

Its limitation is equally established: residual lymphoid tissue creates a long term possibility of recurrent obstruction, recurrent infection, and revision surgery. The absolute risk is usually small in experienced programs but is not uniform, and younger age, operative depth, indication, comorbidity, and institutional technique all appear capable of modifying it.

Extracapsular tonsillectomy therefore remains indispensable. It remains particularly compelling when infection is the dominant problem, when recurrent peritonsillar disease is present, when complete pathological assessment is required, or when the family places a high value on minimizing any possibility of later tonsillar revision. Intracapsular surgery is particularly compelling when obstruction is the dominant problem and reducing pain, hemorrhage, and recovery burden is prioritized.

The contemporary controversy is thus no longer whether intracapsular tonsil surgery is effective. It is how to identify the child in whom the acute morbidity saved by capsule preservation outweighs the small long term price of leaving viable tonsillar tissue behind. That is a more precise question, and it is the question that should shape both operative counseling and the next generation of pediatric tonsil research

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CHAPTER 9

Current Approaches to the Assessment of Gingival Thickness: From Clinical Methods to Ultrasonography

Metehan Ozdemir[1]
Sefa Aydındogan[2]

[1] DDS, MSc,, Selcuk University, Faculty of Dentistry, Department of Periodontology, Konya, Turkey ORCID: 0000-0002-5887-5665
[2] DDS, MSc, Asst. Prof., Selcuk University, Faculty of Dentistry, Department of Periodontology, Konya, Turkey ORCID: 0000-0003-2980-2691

1. Introduction

Gingival thickness is a key parameter that reflects the morphological characteristics of periodontal soft tissues and plays an important role in treatment planning and the prediction of clinical outcomes across various dental procedures. Gingival thickness may vary among individuals and may also differ according to tooth type and anatomical location within the same individual. Differences in the response of thin and thick gingival tissues to inflammatory processes, surgical procedures, and various dental treatments further emphasize the clinical relevance of this parameter (Malpartida-Carrillo et al., 2021).

Gingival thickness is also a key component of the current definition of periodontal phenotype. Following the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions, the term "phenotype" was recommended instead of "biotype," and periodontal phenotype was defined as the combination of gingival phenotype and bone morphotype. Gingival phenotype comprises gingival thickness and keratinized tissue width, whereas bone morphotype is related to alveolar bone thickness (Malpartida-Carrillo et al., 2021). In this context, reliable assessment of gingival thickness is important not only for defining the gingival phenotype but also for identifying individual characteristics of the periodontal tissues.

Gingival thickness can be assessed using clinical methods, such as visual examination, periodontal probe transparency, and transgingival probing, as well as imaging modalities, including cone-beam computed tomography and ultrasonography. These methods differ in terms of invasiveness, measurement accuracy, reproducibility, and clinical applicability. Therefore, selecting an appropriate method is important for the reliable and reproducible assessment of gingival thickness (H. K. Shah, Sharma, & Shrestha, 2020).

2. Clinical Significance of Gingival Thickness

Gingival thickness may vary among individuals, across different tooth groups, and according to anatomical location, and these variations have been associated with the clinical characteristics of periodontal tissues (Kim, Bassir, & Nguyen, 2020).

Thin and thick gingival phenotypes differ in their clinical characteristics. Current evidence indicates that gingival recession may occur more frequently in areas with a thin gingival phenotype and that the extent of recession may be greater than in areas with a thick phenotype (Kim et al., 2020).

However, the presence of a thin gingival phenotype alone does not necessarily indicate compromised periodontal health. Periodontal health can be maintained when good oral hygiene is achieved and iatrogenic factors are absent (Kao et al., 2020).

The relationship between gingival phenotype and alveolar bone thickness is another factor supporting the clinical significance of gingival thickness. A recent systematic review and meta-analysis reported that alveolar bone thickness was significantly greater in areas with a thick gingival phenotype than in those with a thin phenotype. However, the relationship between soft tissue thickness and alveolar bone thickness was not consistently demonstrated across all studies, indicating that the available evidence remains inconclusive in this regard (Shafizadeh, Amid, Tehranchi, & Motamedian, 2022).

The clinical significance of gingival thickness is not limited to periodontal treatment but is also considered in the planning of restorative, implant, and orthodontic treatments. Thin and thick gingival phenotypes may exhibit different tissue responses to inflammatory and traumatic stimuli. Gingival thickness has also been reported to be relevant to the clinical outcomes of periodontal and restorative treatments and to the assessment of peri-implant soft tissues (Abraham, Deepak, Ambili, Preeja, & Archana, 2014). Therefore, reliable and reproducible assessment of gingival thickness before treatment is important for identifying individual tissue characteristics and planning an appropriate treatment approach (Furtak, Leszczyńska, Sender-Janeczek, & Bednarz, 2018).

3. Methods Used for the Assessment of Gingival Thickness

In addition to methods based on clinical examination, various techniques that allow direct measurement or imaging of gingival thickness are used for its assessment. Some of these methods are based on classifying the gingival tissue as having a thin or thick phenotype, whereas others allow gingival thickness to be measured in millimeters. These techniques differ in terms of invasiveness, measurement accuracy, reproducibility, and clinical applicability (Malpartida-Carrillo et al., 2021).

Clinical methods such as visual examination, periodontal probe transparency, and transgingival probing, as well as imaging methods such as cone-beam computed tomography (CBCT) and ultrasonography, are used to assess gingival thickness (Kan, Morimoto, Rungcharassaeng, Roe, & Smith, 2010; R. Shah, Sowmya, & Mehta, 2015; Shao et al., 2018). Although clinical methods have the advantage of being easy to perform, some may be limited by observer-dependent assessment or the need for an invasive procedure. Therefore, different methods have been developed to achieve accurate and reproducible assessment of gingival thickness, and their reliability has been investigated in various studies (Memon, Patel, Sethuraman, Patel, & Arora, 2015).

3.1. Visual Assessment

In visual assessment, the gingival phenotype is determined based on the clinical appearance and morphological characteristics of the gingiva. Gingival tissues with a delicate and more translucent appearance are classified as a thin

phenotype, whereas denser and more fibrous tissues are classified as a thick phenotype (Eghbali, De Rouck, De Bruyn, & Cosyn, 2009). Although visual assessment is a simple and non-invasive method, the evaluation may vary among clinicians.

Limitations in the accurate classification of gingival phenotype have been reported with visual assessment, particularly in the identification of the thin phenotype (Eghbali et al., 2009).

3.2. Periodontal Probe Transparency

Periodontal probe transparency (TRAN) is a non-invasive and easily applicable clinical method used to determine the gingival phenotype. In this method, a periodontal probe is inserted into the gingival sulcus, and its visibility through the gingival tissue is assessed. If the probe is visible through the gingival margin, the phenotype is classified as thin; if it is not visible, the phenotype is classified as thick (De Rouck, Eghbali, Collys, De Bruyn, & Cosyn, 2009). Although this method has the advantages of not requiring local anesthesia and being easy to perform in clinical practice, it does not provide a quantitative measurement of gingival thickness in millimeters (Vlachodimou, Fragkioudakis, & Vouros, 2021).

Phenotype probes with different colored tips have also been developed based on the principle of probe transparency. With these probes, the phenotype can be classified as thin, medium, thick, or very thick according to the visibility of the white, green, and blue tips through the gingival tissue. Nisanci Yilmaz et al. reported a strong correlation between the gingival phenotype determined using color-coded probes and gingival thickness measured by transgingival probing (Nisanci Yilmaz et al., 2022). However, inter-examiner agreement in assessments based on probe visibility may vary depending on the characteristics of the probe used. Uysal et al. reported that although acceptable agreement was observed among assessments performed with different probe types, misclassification may occur when gingival phenotype is determined based on probe visibility. Therefore, both examiner-related factors and the characteristics of the probe should be considered when interpreting assessments based on this method (Uysal, Köse, & Gürkan, 2025).

3.3. Transgingival Probing

Transgingival probing is an invasive method used for the direct measurement of gingival thickness. Following local anesthesia, a periodontal probe, endodontic instrument, or similar thin measuring instrument is inserted perpendicular to the gingival surface until it contacts the underlying hard tissue, and the penetration depth is recorded as gingival thickness. This method is frequently used in clinical studies because it allows gingival thickness to be measured in millimeters (Vandana & Savitha, 2005). However, the need for tissue penetration and local

anesthesia are the main disadvantages of this method. In addition, the angulation of the measuring instrument, the pressure applied to the tissue, and the standardization of the measurement site may affect the values obtained. Therefore, the reliability of transgingival probing is closely related to the standardization of the measurement protocol (Fu et al., 2010).

3.4. Cone-Beam Computed Tomography

Cone-beam computed tomography (CBCT) is an imaging method that allows three-dimensional and cross-sectional evaluation of periodontal tissues. In addition to measuring gingival thickness at specific reference points on CBCT images, the ability to assess alveolar bone structure provides the advantage of evaluating periodontal soft and hard tissues together. Nisanci Yilmaz et al. reported a high level of agreement between gingival thickness measurements obtained using CBCT and those obtained by transgingival probing in the maxillary anterior region (Nisanci Yilmaz et al., 2022).

CBCT measurements may be influenced by imaging parameters such as voxel size and image resolution. In addition, CBCT has certain limitations in visualizing very thin mineralized structures, and its use of ionizing radiation restricts repeated measurements, particularly during short-term follow-up periods (Aydindogan et al., 2026).

3.5. Ultrasonography

Ultrasonography is a non-invasive imaging method that can be used to assess gingival thickness. The technique is based on transmitting high-frequency sound waves into the tissues and evaluating the signals reflected from different tissue interfaces. This allows gingival thickness to be measured without disrupting tissue integrity (N. Shah, Bansal, & Logani, 2014).

The absence of ionizing radiation and the ability to perform repeated measurements are important advantages of ultrasonography (Eghbali, De Bruyn, Cosyn, Kerckaert, & Van Hoof, 2016; Tattan et al., 2020). In addition, the ability to obtain quantitative measurements of gingival thickness may provide a more detailed assessment than clinical classification methods. However, measurement results may be influenced by the characteristics of the ultrasound system, probe positioning, and operator-related factors (Furtak et al., 2018).

3.5.1. Ultrasonographic Assessment of Gingival Thickness

Early applications of ultrasonography for the assessment of gingival thickness primarily used ultrasound systems that provided numerical measurements of soft tissue thickness. With technological advances, high-frequency intraoral ultrasound systems have been developed, allowing cross-sectional imaging of periodontal soft and hard tissues and the evaluation of different anatomical structures within the same image (Tattan et al., 2020). Tattan et al. evaluated

periodontal soft tissue dimensions and alveolar bone levels using a 24-MHz ultrasound probe developed for intraoral use. In their study, soft tissue thickness around the teeth was measured at sites 2 and 5 mm apical to the gingival margin, and the ultrasound measurements were compared with direct clinical measurements. No significant difference was found between ultrasound and direct measurements of soft tissue thickness (Tattan et al., 2020).

Sabri et al. compared gingival thickness measurements obtained using ultrasonography, CBCT, and transgingival probing with histological measurements. The mean gingival thickness was 1.34 mm on histological assessment and 1.25 mm on ultrasonography. Among the methods evaluated, ultrasonography showed the highest agreement with histological measurements. These findings support the potential of ultrasonography as a reliable method for the non-invasive and quantitative assessment of gingival thickness (Sabri et al., 2025).

4. Conclusion

Accurate and reliable assessment of gingival thickness is important for identifying the characteristics of periodontal tissues and planning an appropriate treatment approach. The methods used for this purpose have different advantages and limitations in terms of clinical applicability, invasiveness, and measurement characteristics.

Ultrasonography represents a promising approach because it does not involve ionizing radiation, provides high-resolution cross-sectional images, and allows tissue thickness to be measured without invasive procedures. With these advantages, ultrasonography may serve as an alternative to existing methods for the assessment of gingival thickness and may become more widely used in clinical practice.

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CHAPTER 10

Pregnancy and Radiation

Hatice Bilge Becerir[1]

[1] Prof. Dr., İstanbul Üniversitesi Onkoloji Enstitüsü, ORCID:0000-0002-7688-125X

INTRODUCTION

Each year, numerous women are exposed to ionizing radiation during pregnancy due to medical procedures. Ionizing radiation is known to have harmful effects on human health. As a general rule, the sensitivity of tissue to radiation is directly related to the rate of tissue proliferation. Considering that the human fetus develops from a single cell into an organism in nine months, it is clear that it will be very sensitive to exposure to ionizing radiation. Statistical analysis results of people exposed to the atomic bomb and experimental animal studies have proven that the fetus is sensitive to radiation (Lowe, 2004; International Commission on Radiological Protection [ICRP], 2000). The risk of the fetus being harmed by radiation depends on the dose received and the gestational age (Nakagawa et al., 1997). Radiation has two different effects on the fetus: the deterministic effect of radiation and the stochastic effect of radiation.

Deterministic (Non-Stochastic) Effects of Ionizing Radiation on Fetus

These are the effects that cause death, malformation, growth retardation, and intellectual developmental delay in the fetus or embryo. A threshold (initial) dose is needed for the effects of ionizing radiation to occur; effects do not appear without exposure to this dose. The deterministic effect that will occur with increasing dose increases with increasing dose, as seen in Figure 1. According to the reports prepared by the International Commission on Radiological Protection (ICRP) in 2003 and 2007, doses below 100 mGy = 10 cGy have no significant effect on humans (International Commission on Radiological Protection [ICRP], 2003, 2007). Normally, this dose is definitely not reached in diagnostic medical applications (Baciak & Kearfott, 2000; National Council on Radiation Protection and Measurements, 1989; Osei & Faulkner, 2000; Sharp et al., 1998; Duke University Medical Center, 2009; Parry et al., 1999; Damilakis et al., 2002).

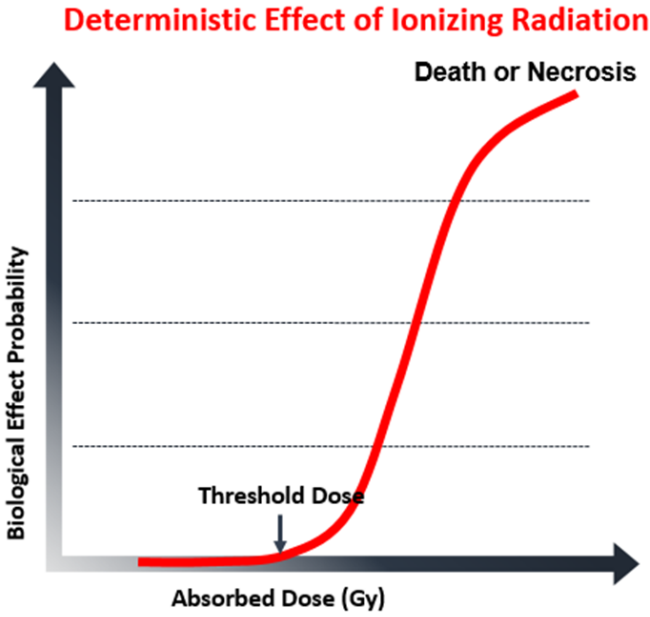


Figure 1. Deterministic Effect of Ionizing Radiation

In diagnostic irradiations, the dose is received directly from the primary radiation beam, from scattered radiation from the collimator in the device head, and from radiation scattered within the tissue. As the primary radiation field, which is directly exposed to radiation, moves away from the pelvic region, the dose received by the patient decreases rapidly; the patient does not receive a dose from the primary radiation field, but only a very low dose from scattered radiation within the tissue. For example, when direct films are taken from areas outside the fetus, the dose received by the fetus is very low. The dose received by the fetus is the same between a chest AP film and a head and neck film: 0.001 cGy.

The area where fluoroscopy is performed on a patient is important. If the fluoroscopy area is outside the fetus, the dose received by the fetus is low. If the area is close, the dose becomes significant. For example, in a fluoroscopy, the dose is 1220 mrad/minute at the center of the field and 7 cm deep, while the dose is 580 mrad/minute at the edge of the field, 10 cm away and 7 cm deep. Time is important in fluoroscopy. When performing a special diagnostic procedure such as angiography, fluoroscopy and radiography methods are used together. If the film taken from the patient is from the pelvic region, the values specified for a direct pelvic film are used. There is no single value specified for angiography. Covering the patient's abdominal area with a lead apron during the examinations does not actually have a dose-reducing effect. The lead apron can reduce the dose

if the radiation comes from outside. Collimator scattering is very small in diagnostic devices. It is not possible to reduce scattering coming from inside the tissue with an externally placed attenuator.

Placing a lead apron on the patient's abdominal area can provide psychological comfort. If the radiation exposure is for radiography purposes and outside the pelvic region, the dose is very small. For example, when a patient undergoes a computed tomography scan of the pelvic region, if the fetus is within the field of view, the average dose is 20 mGy = 2 cGy = 2 rad, while if the fetus is not within the field of view, the dose is 10 mGy = 1 cGy = 1 rad. Even if the irradiated area is the pelvic region, unless it is repeated many times and fluoroscopy is performed for a very long time, exposure to a dose exceeding 100 mGy = 10 cGy = 10 Rad is not possible. If diagnostic imaging is performed using radionuclides, the dose received depends on the type of radionuclide. As can be seen in Table I, the doses received in nuclear applications are quite low. In technetium applications, the dose received by the fetus is less than 5 mGy = 0.5 cGy = 0.5 rad. In technetium applications used for diagnostic purposes, the dose received by the fetus is very small (Baciak & Kearfott, 2000; Osei & Faulkner, 1999).

In recent years, with the decrease in the age at which women are diagnosed with cancer, the number of women diagnosed with cancer during pregnancy has also increased. In the United States, 4000 women are diagnosed with breast, lymphoma, uterine, and thyroid cancer during pregnancy each year and are treated with ionizing radiation (Stovall et al., 1995). If the patient is exposed to radiation for therapeutic purposes, depending on the treated area, the fetus may receive higher doses, and the likelihood of deterministic radiation effects increases. As the treated area moves further away from the fetus, the dose received by the fetus decreases rapidly. During radiation therapy, since the patient remains in the radiation-emitting device for a much longer time than during radiography, scattered radiation from the head of the device also contributes to the total dose. Although this amount is low, it is beneficial to have the patient wear a lead apron covering the pelvic region to eliminate the scattered dose. The doses received by the fetus as a result of radiation treatment of tumors frequently encountered during pregnancy are summarized in Table II. The data presented in this table are obtained from a literature review (King et al., 1985; Nuyttens et al., 2002; Rincón et al., 2002; Sneed et al., 1995; de Wildt et al., 2009; Ring et al., 2005).

Stochastic (Non- Deterministic) Effects of Ionizing Radiation on Fetus

These are effects that do not directly affect the development of the fetus or embryo, but require a period of time called the latent period for the effect to manifest. These effects increase the probability of the person developing cancer or a hereditary disease after birth. There is no threshold (initial) dose required for these effects to occur. The probability of these effects occurring increases

proportionally with the dose. The dose-dependent increase of stochastic effects is shown in Figure 2. When considering the stochastic effects of radiation, it is important to consider the natural frequency of these effects and their variation over the years. Numerous epidemiological studies have been conducted to determine the probable effect of prenatal radiation on childhood cancer development. In these studies, prenatal radiation exposure was found to be associated with an increased frequency of cancer development, with the Relative Risk (RR) ratio of all cancers being between 1.5 and 2.4 (Stewart et al., 1958; MacMahon, 1962).

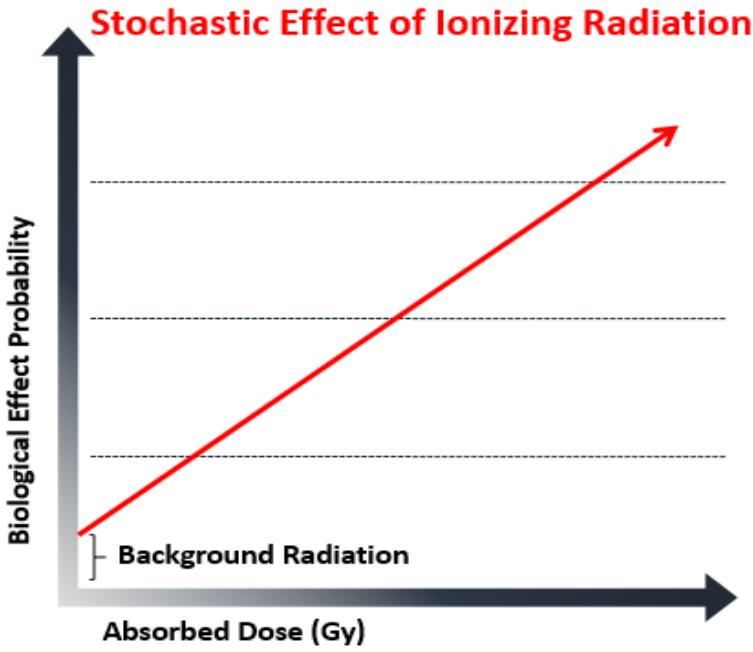


Figure 2. Stochastic Effect of Ionizing Radiation

The largest study in this field is the Oxford Survey of Childhood Cancer (Stewart et al., 1958). According to this study, the RR for leukemia in children up to 10 years of age was stated as 1.92 in those exposed to abdominal irradiation and 1.19 in those exposed to non-abdominal irradiation. In the study conducted by MacMohon, no significant relationship was found between radiation dose and deaths from leukemia (MacMahon, 1962). This does not mean that there is no relationship between the development of leukemia and radiation dose; improvements in leukemia treatments may have caused the mortality rates not to increase. An increased risk was detected in the prospective study conducted by Diamond et al. (1973). As can be understood from the results of the studies, all researchers agree that there is no specific dose value and associated risk increase

amount between prenatal radiation and cancer development (Court Brown et al., 1960; Doll & Wakeford, 1997; Kusama, 1996).

The biggest challenges in conducting such studies are the time lag between radiation exposure and cancer development, the inability to accurately determine the amount of radiation dose received, the inadequacy of records, and the fact that natural cancer incidence rates vary depending on the country, sometimes the region, and even the year, making the evaluation of epidemiological studies difficult.

Relationship between the Effect of Ionizing Radiation and Gestational Age: Pregnancy consists of three stages**.**. The first trimester (first three months) , The second trimester (second three months), The third trimester (last three months).

Days 0-14 (Preimplantation period) : Many women are unaware of the difference between the day they conceive and the day they are exposed to radiation. This period is when they are most sensitive to radiation. The all-or-nothing effect principle is believed to apply during the first 14 days of pregnancy. According to this principle, if the fertilized cell had been exposed to radiation, it would have died like any other cell in our bodies, and pregnancy would not have occurred; since it did occur, it is assumed that the radiation did not hit that cell.

weeks 2–7 (Organogenesis): Organogenesis (organ formation) is the most critical and delicate phase of pregnancy, during which the basic structures of all the baby's major organ systems are established. Organogenesis is also the time when the baby is most vulnerable to external factors (teratogens). Because organs are rapidly forming during this period, factors such as alcohol, smoking, drugs, and radiation can lead to permanent structural abnormalities. Folic acid supplementation is vital during this period for cell division and neural tube closure.

Weeks 8-15: This is the "Transition from Embryonic to Fetal Period" and "From First Trimester to Second Trimester" phase in pregnancy. It marks the beginning of the most comfortable period, when the baby's organ development is complete and it begins to grow rapidly, while the mother adapts to pregnancy.

Weeks 16–25: It is the period when the growth rate slows down and important organ systems begin to form. It is thought that exposure to radiation during this period may cause congenital malformations (Nakagawa et al., 1997; Parry et al., 1999).

Week 26–delivery : The third trimester (last three months) of pregnancy refers to the transition to the new pregnancy stage and the baby's preparation for the world outside the womb. Medically, this period is one of the most critical turning points in terms of the baby's viability and organ maturation. (last three months) of pregnancy refers to the transition to the new pregnancy stage and the

baby's preparation for the world outside the womb. Medically, this period is one of the most critical turning points in terms of the baby's viability and organ maturation. It is believed that even higher doses will not cause organ malformations during this period. The doses received during therapeutic radiation exposure are much higher than those received for diagnostic purposes. The relationship between the effects of radiation and gestational age is summarized in Table I and dose limits based on gestational age is summarized Table II. These tables is based on the study by Wagner et al. (1997).

Tablo I. The relationship between the effects of radiation and gestational age.

Fetal Effect	0-14 days	2-8 weeks	8-15 weeks	15-25 weeks	>25 weeks
Fetal	+++	+	-	-	-
Malformation	-	+++	±	±	±
Mental Retardation	-	-	+++	++	-
Growth Retardation	-	+	+	+	+
Carcinogenic Effect	-	+	+	+	+

- ; No effect observed.
± ; Almost no effect is observed.
+ ; The effect is observed.
++; The effect is clearly observable.
+++; The effect will most likely be observed.

Table II. Dosage lmits based on gestational age

Gestational Age	< 50 mSv	50 – 150 mSv	>150 mSv
< 14 days	Continued is recommended	Continued is recommended	Continued is recommended
2 – 8 weeks	Continued is recommended	Termination is considered (other risks should also be taken into account).	Termination is considered (other risks should also be taken into account).
8 – 15 weeks	Continued is recommended	Termination is considered (other risks should also be taken into account).	High risk, but termination may not be necessary.
> 15 weeks	Continued is recommended	Continued is recommended	Continued is recommended

Fetal Exposure to Radiation for Diagnostic Purposes:

Diagnostic doses are usually administered during the first trimester. The person is unaware of their pregnancy. To minimize exposure to diagnostic radiation, the procedure should be performed on days when fertility is not possible in women of childbearing age. Deciding to terminate a pregnancy after radiation exposure is not easy. The decision to terminate should be made considering the radiation dose, other risks to the expectant mother, the mother's age, and her general health status. Doses received in basic diagnostic applications are summarized in Table III. The applications and doses received in Table III are data obtained as a result of literature research (National Council on Radiation Protection and Measurements, 1989; Baciak & Kearfott, 2000; Osei & Faulkner, 2000; Sharp et al., 1998; Duke University Medical Center, 2009; Parry et al., 1999; Damilakis et al., 2002).

Table III. Doses received in basic diagnostic applications

APPLICATION	mSv	cGy
Radiography		
Head and Neck	0.011	0.0011
Thorax	0.011	0.0011
Gastrointestinal	1	0.1
Abdomen	2.5	0.25
Intravenous Pyelography (IVP)	4.8	0.48
Urinary System	3.9	0.39
Mammography	0.5	0.05
Bone density	2.5	0.25
Dental X-ray	0.001	0.0001
Computed Tomography		
Head and Neck	0.01	<0.01
Thorax	1.2	0.12
Abdomen (Fetus in the field)	25	2.5
Nuclear Medicine		
Tc-99 Bone scan (fosfat MBq)	<5	<0.5
I-131 Troid (0.55 MBq)	<0.04	<0.004

There is no evidence that exposure of the fetus to doses below 50 mGy = 5 cGy = increases congenital malformations. Two cases were presented in the study published by Nuyttens et al. (2002). In the first case, a 26-year-old patient was irradiated with 6 MV high-energy X-rays, 19 Gy, in 12 fractions, in the mediastinum region at 27 weeks of gestation with a diagnosis of Hodgkin lymphoma. The estimated dose received by the fetus was a maximum of 42 cGy and a minimum of 11 cGy. The baby was born without problems, and after 8 years of follow-up, no malformations or growth retardation were observed, and the mother continues to live without disease. In this case, it was thought that the fetus's head was downward and that it was 27 weeks of gestation at the time of

irradiation were the biggest factors in the absence of deterministic effects of radiation.

In the second case, a 29-year-old patient was diagnosed with left oral cavity cancer and was irradiated with 50 Gy, 25 fractions of 6 MV high-energy X-rays in the 22nd week of her pregnancy. Seven weeks after treatment, a healthy baby was born. In contrast to this study, King et al.(1985) study reported that 20 patients with breast cancer received radiation therapy and perinatal death was reported in 3 cases (2 cases due to hypoxia, 1 case due to intrauterine death). Estimated doses received by the fetus were not reported (Nuyttens et al., 2002).

When evaluating two studies with conflicting results, it is quite difficult to draw a definitive conclusion. However, it can be said that if patients have to undergo radiation therapy, it is appropriate to postpone the application as much as possible until the period when the fetus is more resistant to radiation. It should not be forgotten that every 1 cGy dose increases the risk of developing childhood tumors and that every 1 Gy = 100 cGy radiation dose increases the actual risk by 6% for childhood cancers (Doll & Wakeford, 1997).

An observational prospective cohort study was conducted on pregnant women who applied to the Teratology Information Service of Careggi University Hospital in Florence (Italy) for teratogenic risk counseling between 2005-2015 (Clementi et al., 2021). The study excluded individuals at risk of spontaneous miscarriage, those with multiple miscarriages, drug and alcohol users, those carrying immunodeficiency virus, and those using teratogenic drugs. Pregnant women included in the study were those exposed to diagnostic radiation. Individuals exposed to radiation through nuclear medicine procedures were not included in the study.

The participants were divided into two groups based on the area of radiation exposure. The first group consisted of those exposed to radiation in the abdominal and lumbar regions (130 pregnant women), and the second group consisted of those exposed to radiation in body parts other than the abdominal and lumbar regions (415 pregnant women). In addition to these two groups, a control group (1434 pregnant women) was randomly selected from patients who presented to the teratology unit without radiation exposure during pregnancy. Those exposed to radiation doses less than 1 mSv were considered risk-free, so no calculations were performed. Data included births before 37 and 34 weeks, birth weight, head circumference (cm), and postnatal Apgar scores at 1 and 5 minutes. According to the study results, the rate of elective pregnancy termination (before 90 days) was 10.8% in those exposed to radiation in the abdominal and lumbar regions, 5.1% in those exposed to radiation outside the abdominal and lumbar regions, and 3.3% in the control group who were not exposed to radiation. The rate of terminating pregnancies after 90 days for medical reasons was 2.3% in those exposed to abdominal and lumbar radiation, while it was below 1% in the other two groups.

There was no statistically significant difference in the incidence of congenital anomalies for all three groups. The incidence was 3.1% in those exposed to radiation in the abdominal and lumbar regions, 1.5% in those exposed to radiation outside the abdominal and lumbar regions, and 1.7% in the control group. When the study results were evaluated according to the amount of radiation dose received, the live birth rate was 75.5% in those exposed to radiation doses less than 20 mSv in the abdominal and lumbar regions, 41.7% in those exposed to 20-50 mSv, and 40% in those exposed to 50 mSv. ccording to the study, women exposed to radiodiagnostic procedures involving the maternal thyroid (i.e., head, teeth, neck, and chest X-rays) had a higher rate of small-for-gestational-age babies. The possible role of radiation involving the maternal thyroid gland in the occurrence of small-for-gestational-age babies, particularly among male newborns, is still debated, although it has been reported in previous studies.

The comprehensive study confirms that exposure to radiodiagnostic procedures at doses below 100 mSv that may affect the uterus does not increase the risk of significant effects, as previously shown in earlier studies, and that although obtained in a small number of cases, maternal thyroid radiation exposure during the first trimester of pregnancy increases the risk of low birth weight.

Fetal Exposure to Radiation for Radiotherapy Purposes:

The doses administered during treatment are generally during the second and third trimesters. The time it takes to make a diagnosis, perform all necessary tests, and establish a treatment protocol causes the gestational age to advance.

Cancer diagnosis during pregnancy is not very common. It is a rare event, occurring in 1 out of 1000 pregnancies. The most common cancer during pregnancy is breast cancer (1 in 3000 pregnancies), followed by Hodgkin's disease, melanoma, and leukemia. Radiotherapy is the primary treatment for the first three. The incidence of breast cancer diagnosed during pregnancy increases with the age of the pregnant woman. In treatment protocols, radiotherapy is planned after surgery and chemotherapy, usually during the second or third trimester of pregnancy. If possible, it is preferred to be done after delivery. In breast cancer radiotherapy, a dose of 50 Gy is usually prescribed to the chest wall. The dose received by the fetus is 5-15 cGy. In some cases, it can reach 20 cGy.

In gynecological tumors, the decision is whether or not to perform radiotherapy because irradiation of the pelvic region is necessary. If radiotherapy is decided upon, the pregnancy is terminated. The International Federation of Gynecology and Obstetrics (FIGO) advocates for the termination of pregnancy until stage IIB. In head and neck radiotherapy, doses are lower due to the distance from the fetus. The average dose received is around 402 cGy. In Hodgkin's and Non-Hodgkin's lymphomas, the prescribed dose is 20-30 Gy. The dose received by the fetus is estimated to be 13-15 cGy.

Treatment planning systems used for dose calculations in radiotherapy applications do not calculate doses outside the 20 cm radius of the treatment field. This often makes it difficult to calculate the dose received by the fetus, which is located outside the treatment field. Advanced treatment techniques used today aim to protect critical organ doses. Techniques such as intensity-modulated radiotherapy, volumetric arc radiotherapy, and neurotactic radiotherapy can keep fetal doses within limits. The entire team must be informed of the situation (Gueiderikh et al., 2025).

Fetal exposure to radiopharmaceutical treatments:

Estimates regarding fetal dose are based on phantom studies and animal studies. Fetal dose estimates can be made using calculation methods. Radiopharmaceuticals are not administered if the patient is known to be pregnant. The exception to this is suspected pulmonary embolism during pregnancy. Fetal overdose is not a concern in diagnostic procedures. There is no indication for therapeutic use of radiopharmaceuticals (Stabin, 2017).

CONCLUSION

The fetus is sensitive to ionizing radiation and the effects of radiation depend on the radiation dose and gestational age. The risk is not the same during all periods of pregnancy. Especially during the early stages of pregnancy and organogenesis, the developing embryo is more sensitive to radiation. For this reason, gestational age should always be considered when evaluating radiation exposure during pregnancy.

Diagnostic radiation procedures generally result in low fetal doses, especially when the irradiated area is far from the fetus. Radiotherapy is different from diagnostic radiology because the radiation doses are much higher. The fetal dose depends on the treatment site, prescribed dose, distance between the fetus and treatment field, and scattered radiation. If possible, radiotherapy should be postponed until after delivery. When radiotherapy cannot be postponed, careful treatment planning and fetal dose estimation are necessary. The decision should be made together by radiation oncologists, medical physicists and obstetricians. Radiopharmaceutical applications also require evaluation of fetal exposure according to the type of radiopharmaceutical and the purpose of the procedure. Although fetal exposure is generally not a major concern in diagnostic nuclear medicine procedures, therapeutic radiopharmaceutical applications require special consideration during pregnancy.

In conclusion, medical procedures involving ionizing radiation should be avoided during pregnancy. If necessary, appropriate protection should be provided and fetal risk assessed. The most important factors in assessing fetal risk are radiation dose, gestational age, and site of radiation exposure.

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CHAPTER 11

Inflammatory Processes in Renal Ischemia and the Role of Flavonoids

Rasim Mogulkoc [1]
Abdulkerim Kasim Baltaci[2]

[1] Dr., Selcuk University, Medical School, Department of Physiology, Konya, TURKEY
Correspondence to
[2] Selcuk University,Medical School, Department of Physiology
Konya-TURKEY

Introduction

Renal ischemia-reperfusion (I/R) injury is recognized as a well-defined model of acute kidney injury that develops following the restoration of blood flow after a temporary cessation of renal blood flow (Yang et al., 2017). Clinically, the consequences are significant as it arises in commonly encountered situations such as partial nephrectomy, cardiovascular surgery, renal transplantation, trauma, and shock (Peng et al., 2023). During the ischemic phase, ATP production decreases due to a lack of energy substrates and oxygen; a loss of structural integrity begins to occur, particularly in proximal tubule cells (Pan and Zhu, 2026). In the reperfusion phase, tissue repair initiates alongside reoxygenation; however, an increase in reactive oxygen species (ROS) can exacerbate secondary injury through endothelial activation (Zhang et al., 2022). In short, renal I/R injury is not merely the result of transient hypoperfusion but is a multi-component process involving the interplay of oxidative stress, microvascular dysfunction, and inflammation (Peng et al., 2023). Oxidative stress and inflammation are two key components playing fundamental roles in both the pathogenesis and progression of acute kidney injury (AKI) (Peng et al., 2023). NF-κB and Nrf2 are the key transcription factors that regulate inflammatory and oxidative stress responses, respectively, following AKI. During reperfusion, tissue reoxygenation intensifies oxidative stress by increasing free radical production (Peng et al., 2023). Comparisons of rat and mouse models subjected to varying durations of ischemia and reperfusion have shown that renal I/R injury causes acute kidney failure by increasing oxidative stress and inflammation in renal tissue (Güvenç et al., 2020). The energy crisis occurring during the early phase of I/R injury is associated with a loss of mitochondrial membrane potential, intracellular Ca2+ accumulation, and the activation of apoptotic and necrotic cell death pathways (Pan and Zhu, 2026). In the late phase, the amplification of the innate immune response, the release of pro-inflammatory mediators, and leukocyte infiltration drive the persistence of tissue damage (Meng et al., 2020). The activation of the TLR4-NF-κB axis and the subsequent rise in IL-1β and IL-6 levels indicate that these signaling pathways lie at the core of the inflammatory response (Meng et al., 2020). Activation of the Nrf2/HO-1 axis serves as a protective counter-regulatory mechanism that mitigates tubular damage and lipid peroxidation by enhancing cellular antioxidant capacity (Wei et al., 2025). The reperfusion phase is regarded not merely as the restoration of oxygen supply, but as a secondary injury phase characterized by a convergence of ROS elevation, pro-inflammatory mediator release, the activation of apoptosis-related genes, and Ca2+ overload. Consequently, the early intervention window in I/R injury is a critical determinant of clinical outcomes (Mohammed and Rizij, 2025).

AKI is a clinical condition characterized by a sudden decline in renal function, leading to a disruption of internal homeostasis, retention of uremic toxins, and

multi-system involvement—particularly affecting the immune, cardiovascular, and neurological systems (Mohammed and Rizij 2025).

Renal injury primarily develops in the tubules and is characterized by ischemia-induced tubular necrosis (Pan and Zhu 2026). In post-ischemic acute kidney injury, observations include congestion, cloudy swelling of the tubular epithelium, acute tubular necrosis, inflammatory cell infiltration, a decrease in the glomerular filtration rate, edema, and increased resistance in renal vessels (Güvenç et al. 2020).

The Notch signaling pathway is a cell-to-cell communication mechanism that regulates developmental processes—such as lateral inhibition, boundary formation, and cell fate decisions—as well as regeneration and homeostasis in adult tissues (Mohammed and Rizij 2025). Following ligand-receptor interaction, sequential proteolytic cleavage releases the Notch intracellular domain (NICD), which translocates to the nucleus and upregulates the transcription of target genes (Mohammed and Rizij 2025). While basal Notch activity is limited in the adult kidney, it has been reported that this pathway can be reactivated during acute or chronic kidney injury; furthermore, increased expression of processed Delta-1, Notch-2, and Hes-1 in renal ischemia/reperfusion (I/R) models may be associated with tubular epithelial proliferation (Mohammed and Rizij 2025).

A study by Dong et al. (2025) demonstrates that silibinin attenuates renal ischemia-reperfusion injury (IRI) by modulating PI3K/AKT signaling, inflammation, and oxidative stress. First, the role of PI3K/AKT in renal IRI was investigated, and it was demonstrated that silibinin significantly reduced the levels of MDA, BUN, serum Cr, and H2O2, which were elevated by IRI. Silibinin was shown to reverse the IRI-induced increases in the proinflammatory cytokines IL-1β and IL-6 at both the mRNA and protein levels. While IRI suppressed AKT and PI3K phosphorylation, silibinin reversed this effect. These results indicate that silibinin reduces inflammation and oxidative stress by enhancing PI3K/AKT phosphorylation. Silibinin's ability to increase PI3K/AKT phosphorylation and suppress MAPK (p38, ERK1/2, JNK) phosphorylation is consistent with published evidence regarding the reciprocal cross-regulation between the PI3K/AKT and MAPK pathways. PI3K/AKT activation inhibits upstream kinases and negatively regulates MAPK; conversely, excessive MAPK activation can suppress PI3K/AKT through feedback mechanisms (Dong et al., 2025).

Ischemia-reperfusion (I/R) injury encompasses a range of conditions characterized by acute coronary syndrome (ACS), acute kidney injury (AKI), and circulatory and vascular disorders. It can also occur following organ transplantation or various surgical procedures. I/R can result in numerous metabolic processes within the cell, such as a decrease in ATP levels, an increase in intracellular and mitochondrial calcium levels, alterations in cellular ion and pH levels, endoplasmic reticulum stress, and the generation of reactive oxygen

species (ROS) (Devarajan 2006; Giaccia et al. 2004; Giordano 2005; Kalogeris et al. 2016).

The pathophysiology of I/R injury is extensive and complex. Ischemia involves apoptosis, inflammation, necroptosis, mitophagy, oxidative stress, mitochondrial dysfunction, and damage to signaling pathways (Kalogeris et al. 2016). During ischemia, the reduction in oxygen supply and the shift from aerobic to anaerobic metabolism decrease the rate of ATP production and lead to lactate accumulation, causing intracellular acidosis (Eltzschig and Eckle 2011). Na+/K+-ATPases and Na+/H+ and Ca2+-ATPase pumps fail to function; consequently, sodium, hydrogen, and calcium accumulate in the cytoplasm, leading to hyper-osmolality, increased water transport across cell membranes, and cellular swelling (Wu et al. 2018).

During reperfusion, oxygen and other molecules required for aerobic ATP production reach the organ. Intracellular pH returns to normal thanks to the H+ ions circulating within the cell; however, reperfusion itself can also cause cellular damage. This phenomenon was first observed when reperfusion following coronary ligation led to necrosis in the heart (Jennings et al., 1960). Reperfusion injury is an event that occurs after blood flow is restored to the tissue, rather than during the ischemic period itself. This damage can be completely eliminated or mitigated (Ozden et al., 2001). During reperfusion injury, the rapid increase in oxygen and changes in pH can elevate cytosolic calcium levels by activating cysteine proteases and apoptotic pathways. Excessive calcium overload triggers the opening of mitochondrial permeability transition pores (mPTPs), facilitating the release of damage-associated molecular patterns (DAMPs)—such as cytochrome c, succinate, and DNA—that initiate cell death via apoptosis and necrosis (Krysko et al., 2011; Mills et al., 2017). In addition to these mechanisms, I/R injury can induce epigenetic changes in damaged tissues and organs. Epigenetic changes are alterations occurring at the gene level—mediated by the properties of protein complexes—without changing the DNA sequence itself (Khan and La Thangue, 2012). These include various post-translational modifications such as acetylation, methylation, phosphorylation, SUMOylation, ubiquitination, carbonylation, and glycosylation, as well as DNA methylation (Bolden et al., 2006; Nightingale et al., 2006). It has been suggested that understanding the epigenetic changes triggered by I/R could contribute to future clinical advancements (Tang and Zhuang, 2019). Despite clinical advancements in kidney transplantation, acute complications can occur in some patients post-transplant; among these, ischemia-reperfusion (I/R) injury is a primary cause of delayed graft function (DGF). DGF entails prolonged hospitalization and subsequent serious complications, resulting in significant social and economic impacts. During I/R injury, key pathways are activated, including ferroptosis—a form of cell death characterized by oxidative stress, iron accumulation, and excessive lipid peroxidation—and mitophagy, the selective autophagic

degradation of damaged mitochondria. While ferroptosis contributes to renal injury, mitophagy may play a protective role by reducing the release of reactive oxygen species from dysfunctional mitochondria. Although clinical applications are currently limited, animal studies indicate that modulating both mitophagy and ferroptosis can attenuate I/R injury (Granata et al., 2022). Due to its specialized tissue structure and function, the kidney is particularly susceptible to ischemia-reperfusion injury (IRI). Currently, there are no specific pharmacological treatments for renal ischemia-reperfusion injury. Multiple signaling pathways are implicated in the pathogenesis of this condition; generally, processes such as Ca2+ overload, the release of free oxygen radicals, activation of cell adhesion molecules, chemokine release, and leukocyte interactions are believed to drive tissue inflammation, necrosis, apoptosis, and ultimately, organ failure (Kalyanaraman, 2013; Wang et al., 2013).

Clinically, renal IRI frequently occurs during kidney transplantation, partial nephrectomy, and cardiac surgery. Furthermore, it is a primary cause of acute kidney injury, accounting for 20% of cases among hospitalized patients (Lafrance and Miller 2010). Consequently, effectively preventing renal IRI and reducing mortality have become significant clinical objectives. Briefly, the ischemic phase leads to dysfunction of glomerular capillary endothelial cells and necrosis of renal tubular epithelial cells. Subsequently, the reperfusion phase exacerbates the injury by triggering the release of large amounts of reactive oxygen species and activating various enzymes; a robust inflammatory response is present during both phases (Kinsey et al. 2008). Additionally, both innate and adaptive immune responses are involved in IRI, alongside various programmed cell death pathways (Malek and Nematbakhsh 2015).

Renal IR is regulated by multiple signaling pathways. Renal tubular epithelial cells (TECs) play a pivotal role in the IRI process, acting as key effectors of the injury (Smith et al. 2019). Three key signaling factors associated with IRI in renal tubular epithelial cells have been identified: Toll-like receptors (TLRs), sphingosine-1-phosphate (S1P) receptors, and hypoxia-inducible factors (HIFs) (Akhtar et al. 2014; Koch et al. 2013; Wu and Chadban 2014). TEC signaling pathways involve significant intracellular and intercellular effects, making them potential targets for IRI therapy (Yan et al. 2021).

A study using Sprague-Dawley rats investigated the protective effects of exogenous spermine against renal ischemia-reperfusion injury. Spermine was injected into the tail veins of rats at various doses (2 ml/kg) 30 minutes before and 3 days after renal ischemia-reperfusion. It was concluded that spermine is not toxic to rat kidneys at a dose of 2 ml/kg and a concentration of 200 μmol/L or lower. Spermine doses below 200 μM were observed to reduce renal ischemia-reperfusion injury in rats. It was concluded that spermine alleviates ischemia-reperfusion injury via the Akt/mTOR/HIF-1α/BNIP3 pathway (Yan et al., 2021).

A study using 30 male wild-type C57BL/6 mice investigated the effects of isoquercitrin (IQ) on renal ischemia-reperfusion (RIRI) injury. To induce renal ischemia-reperfusion, the right kidney was removed, the left renal pedicle was clamped for 30 minutes, and this was followed by 24 hours of reperfusion. Following RIRI, levels of BUN, creatinine, TNF-α, IL-6, malondialdehyde, and Bax increased significantly, while superoxide dismutase levels, the Bcl-2/Bax ratio, and Bcl-2 expression decreased markedly. IQ treatment reversed these changes. Consequently, it was demonstrated that IQ may exert protective effects—such as anti-apoptotic and anti-inflammatory actions—against RIRI, thereby alleviating the injury (Liang et al., 2020). A study using mice (aged 9–10 weeks) sought to elucidate the effect of Mint3 on ischemia-reperfusion via the HIF-1 and NF-κB pathways. Mint3 is a molecule that activates HIFs. Kidneys in mice underwent fibrosis following unilateral ischemia-reperfusion injury. After an ischemia duration of 23 minutes, the kidneys were harvested 24 hours and 7 days post-ischemia-reperfusion. Consequently, the degradation of Mint-3 exacerbated tubulointerstitial fibrosis by increasing apoptosis in tubular epithelial cells (TECs). It was found that these effects of Mint-3 were independent of the HIF pathway and were mediated by the anti-apoptotic function of NF-κB (Nasu et al., 2020).

Homeostasis is essential for the maintenance of life. Although free radicals are toxic metabolites for cells, antioxidants work to counteract their effects. Antioxidants can be classified based on their origin or mode of action—for instance, as endogenous vs. exogenous, natural vs. synthetic, or direct vs. indirect. Endogenous antioxidants include glutathione peroxidase, catalase, glutathione reductase, superoxide dismutase, and glutathione, among others. Exogenous antioxidants include molecules such as beta-carotene (vitamin A), folic acid (vitamin B9), and ascorbic acid (vitamin C) (Akpınar 2021). There is a close relationship between reduced tissue and organ function and oxidative stress caused by ROS. Intracellular redox homeostasis depends on the balance between ROS production and ROS scavenging, maintained by antioxidants such as SOD, GPx, GSH, and CAT. Disruptions to this balance cause cellular damage. Excessive ROS production leads to oxidative damage to DNA, lipids, and proteins, while by-products of oxidative stress—such as MDA, a product of polyunsaturated fatty acid peroxidation—accumulate concurrently (Davalli et al. 2016; Feng et al. 2020). ROS and oxidative stress exert pathogenic effects in various bone and muscle diseases, primarily by regulating the NF-κB pathway, the mitogen-activated protein kinase pathway, and the phosphatidylinositol 3-kinase pathway (Min et al. 2019; Morgan and Liu 2011; Schröder 2019).

Under normal physiological conditions, the body can produce small amounts of ROS (Pi et al. 2010). Free radical-scavenging enzymes and antioxidants maintain the balance of oxygen metabolism by activating transcription factors, regulating physiologically active substances and inflammatory immunity, and

promoting cell proliferation and differentiation of physiological importance. However, oxidative stress arises when the balance between the levels of ROS and reactive nitrogen species (RNS) and the antioxidant defense system is disrupted (Chandel et al. 2000). ROS—primarily including the superoxide anion and hydrogen peroxide—are the key elements that initiate oxidative stress in vivo. In cells, significant amounts of ROS are produced by the mitochondrial electron transport chain and the cytochrome P450 family, while enzymes such as xanthine oxidoreductase, NOX, nitric oxide synthase, and other catalytic enzymes heavily influence ROS generation (Sedeek et al. 2013). RNS constitute a class of nitric oxide (NO)-derived species—including NO, nitrogen-oxygen anions, nitrosothiols, and peroxynitrite—produced through the reaction of NO with ROS. Excessive ROS and RNS can damage cellular structure and function by reacting with intracellular lipids, nucleic acids, and proteins, thereby leading to lipid peroxidation, oxidative DNA damage, and intracellular protein denaturation (Martinez-Useros et al. 2017). Oxidants can also act as signaling molecules to alter intracellular signaling pathways and even gene expression (Kim et al. 2002). Additionally, oxidative modification can promote abnormal cell growth, inflammation, and other physiological processes (Barnes and Gorin 2011; Ray et al. 2012). The levels of free radicals can increase in response to environmental factors. Naturally, they regulate vital cellular activities such as mitochondrial activity and cell division. However, under conditions of oxidative stress, they can also cause irreversible damage to the cell's structural components (Akpınar 2021).

All flavonoids possess antioxidant activity due to their 3',4'-dihydroxy configuration. Phenolic antioxidants interact with lipid oxidation processes by rapidly donating H+ ions to lipid radicals. Their function is to scavenge lipid peroxy (ROO•) and alkoxy (RO•) radicals, thereby terminating the lipid peroxidation chain reaction (Çimen 1999). As a small, highly lipid-soluble molecule, DiOHF can rapidly enter the cell and inhibit both intracellular and extracellular ROS. Furthermore, flavonols have been shown to have a plasma half-life exceeding 24 hours, which may facilitate long-term protection. The mechanism underlying flavonoid antioxidant activity is primarily radical scavenging; DiOHF possesses the structural features known to enhance this activity—specifically, a catechol group on the B-ring, a 3-OH group on the C-ring, and a C2-C3 double bond (Chan et al. 2003).

DiOHF exerts inhibitory effects on NADPH oxidase-mediated superoxide formation (Jiang et al. 2008). The effects of 54 highly bioavailable components of the Tibetan medicine formula SHP (Src homology-2 (SH2) domain-containing protein tyrosine phosphatase) were investigated using a renal ischemia-reperfusion model. Analyses revealed that SHP regulates various targets, including TNF-α, IL-6, GAPDH, MMP9, PTGS2, and MMP6; ...has revealed that quercetin, kaempferol, and luteolin bind to these targets with high affinity (Gomchok et al., 2025).

Research findings indicate that SHP provides an innovative mechanism and theoretical basis for RIRI treatment through multiple biological pathways and processes (Gomchok et al., 2025). In mouse models of AKI and the transition from AKI to CKD, MSCs (mesenchymal stem cells) pre-treated with AST improved renal function and reduced inflammation. RNA-seq analysis revealed an upregulation of the PTGS2 pathway in AST-MSCs, while molecular docking and SPR (surface plasmon resonance) analyses demonstrated that AST interacts with KLF4 (Krüppel-like factor 4) to activate the PTGS2 promoter. AST-MSCs promoted the M2 phenotype in RAW264.7 macrophages by increasing PGE_2 (prostaglandin E_2) secretion, whereas PTGS2 deletion reversed these therapeutic effects and macrophage polarization. It was determined that AST pre-treatment enhances the efficacy of MSCs in treating AKI and AKI-CKD by triggering PTGS2-mediated M2 macrophage polarization (Geng et al., 2024). Fisetin reduces myocardial, cerebral, renal, and hepatic I/R-induced cellular damage without causing serious side effects, thanks to its potent antioxidant, anti-inflammatory, mitochondria-protective, autophagy-promoting, and anti-apoptotic properties (Adeli et al., 2024); meanwhile, another study found that the addition of quercetin and sucrose to UW preservation solution lowered AST, ALT, and LDH levels following 30 minutes of reperfusion (Otani et al., 2023). The protective effect of kaempferide, isolated from the *Kaempferia galanga* rhizome, against acute kidney injury (AKI) was investigated in both *in vitro* (HK-2 cells) and *in vivo* (mouse) models. In *in vitro* experiments, 10 μM kaempferide or kaempferol inhibited cisplatin-induced cellular injury. In the *in vivo* model, oral administration of kaempferide (50 mg/kg) before or after a single dose of cisplatin (15 mg/kg, i.p.) reduced tubular necrosis, inflammation, and oxidative stress. These findings indicate that kaempferide is a potent protective agent against cisplatin-induced AKI (Shao et al., 2023). In male Wistar rats, quercetin treatment restored creatinine and MDA levels toward normal following ischemia-reperfusion (IR) injury. It enhanced antioxidant enzyme activities and suppressed the NF-κB pathway and apoptosis-related factors. It also reduced matrix metalloproteinase production. These results demonstrate that a single dose of quercetin mitigates I/R injury through antioxidant, anti-inflammatory, and anti-apoptotic mechanisms (Bagheri et al., 2023).

In another study where AKI was induced in mice via 30 minutes of renal ischemia followed by 24 hours of reperfusion, quercetin-loaded polymeric nanoparticles (10 mg/kg) were administered intravenously one hour later. Quercetin nanoparticles restored elevated BUN, creatinine, and cystatin C levels to normal and significantly reduced histological lesions, lipid peroxidation, and the accumulation of 3-nitrotyrosine and COX-2. These findings indicate that quercetin-loaded polymeric nanoparticles possess therapeutic potential for the *in vivo* treatment of I/R-induced AKI (Huang et al., 2022). When nicorandil or rutin trihydrate was added to a kidney cell preservation solution *in vitro*,

measurements showed improvements in ATP levels, ROS levels, cell viability, and caspase-3 suppression; similarly, *in vivo* studies in mice using the same solutions resulted in improved serum creatinine and BUN levels. These results indicate that supplementation with nicorandil or rutin trihydrate can support cellular functions (Sharma et al., 2022). Male Wistar rats were pre-treated with 20 mg/kg fisetin (i.p.) 30 minutes prior to ischemia-reperfusion, followed by 45 minutes of bilateral renal clamping and 24 hours of reperfusion. It was reported that fisetin significantly attenuated renal ischemia-reperfusion (IR) injury by reducing apoptosis and free radical formation (Prem and Kurian, 2022). In rats subjected to renal ischemia-reperfusion injury (RIRI), the apoptotic index, TOS (total oxidant status), and levels of TNF-alpha, BUN, and urea were found to be significantly lower in the RIRI + oxerutin group. These results demonstrated that oxerutin exerts protective effects against RIRI at both histopathological and biochemical levels. It was noted that prophylactically administered oxerutin reduced RIRI-induced apoptosis and renal failure, suggesting its potential use as an effective protective agent in the treatment of RIRI (Güzel et al., 2022).

Quercetin demonstrated a protective effect against I/R injury by improving renal function and attenuating histopathological changes in both renal and myocardial tissues. While renal kallikrein-kinin system (KKS) content and activity increased, myocardial cystathionine γ-lyase (CSE) levels and hydrogen sulfide (H2S) content were modulated. Furthermore, quercetin increased levels of nitric oxide (NO), nuclear factor erythroid 2-related factor 2 (Nrf2), glutamate-cysteine ligase (GCL), glutathione (GSH), and peroxiredoxin-2 (Prx2) in myocardial tissue, while reducing malondialdehyde (MDA), nuclear factor-kappa B (NF-kB), caspase-3, and Rho-kinase activity. These findings indicate that the cardio-renal protective effects of quercetin are associated with the Rho-kinase pathway and H2S production (El-Sayed et al., 2021). Quercetin exhibited a nephroprotective effect by preventing ischemia-induced glomerular loss, suggesting its potential use as a preventive agent in renal surgery (Gonçalves et al., 2021). Ischemia-reperfusion injury (IRI) impaired renal function and disrupted tissue integrity, thereby increasing inflammation, apoptosis, and oxidative stress; carvedilol treatment attenuated these effects. Pre-injection of quercetin and LiCl enhanced the renoprotective effects of carvedilol against I/R injury, independently of changes in lipid mediators such as phosphatidylinositol 4,5-bisphosphate (PIP2) and diacylglycerol (DAG). Thus, quercetin and LiCl enhance the protective effects of carvedilol—specifically the reduction of inflammation and oxidative stress—in mice fed an HFrHFD (high-fructose, high-fat diet) (Rezk et al., 2021).

The IR mouse model was established by excising the right kidney, followed by clamping the left renal pedicle for 30 minutes and allowing for 24 hours of reperfusion. Following IR, levels of BUN, creatinine, TNF-α, IL-6, malondialdehyde, and Bax (an apoptosis regulator) increased, whereas

superoxide dismutase activity, the Bcl-2/Bax ratio, and Bcl-2 expression decreased; IQ treatment reversed these changes. IQ demonstrates protective effects against IR through antioxidant, anti-inflammatory, and anti-apoptotic mechanisms (Ve et al., 2020). In a rat IR model, intraperitoneal administration of flavonoid-containing APE (at a dose of 30 mg/kg) three days prior to IR induction resulted in a significant reduction of MDA, TNF-α, IL-1β, and NF-kB levels compared to the IR group. These findings indicate that APE is a promising nutraceutical and functional food source for managing complications associated with renal disorders (Yousefi-Manesh et al., 2019). Pre-treatment with hyperoside in mice reduced IR-induced AKI, tubular cell apoptosis, and oxidative stress. Hyperoside inhibited mitochondrial fission by suppressing the OMA1-mediated proteolysis of OPA1. It was also shown to inhibit CoCl2-induced mitochondrial fission, oxidative stress, and apoptosis in human proximal tubular cells by regulating the OMA1-OPA1 axis. These findings indicate that hyperoside possesses therapeutic potential for the treatment of AKI (Lin et al. 2019). RUT treatment reduced intracellular reactive oxygen species and malondialdehyde levels while inhibiting cell apoptosis. RUT reversed the CP-induced increase in p53, cleaved caspase-3, and elevated levels of pro-caspase-3 and pro-caspase-9. Thus, RUT was observed to alleviate CP-induced nephrotoxicity by inhibiting the p53/caspase pathway (Wu et al. 2019). In I/R injury, glutathione peroxidase and catalase activities decreased, while malondialdehyde levels increased and histological damage occurred in kidney and liver tissues. RIPerC and quercetin treatments attenuated these changes, with combined therapy further enhancing recovery. The study demonstrates that quercetin and RIPerC (remote ischemic preconditioning) protect the kidney and liver following I/R injury, reinforcing this protection by reducing lipid peroxidation and boosting antioxidant systems (Gholampour and Sadidi 2018). In an I/R model, quercetin treatment accelerated wound closure and reduced immune cell infiltration and pro-inflammatory cytokine production. Signal analysis revealed that quercetin inhibited MAPK but did not affect NF-κB activation. Quercetin improved wound healing in I/R lesions by suppressing the MAPK (mitogen-activated protein kinase) pathway. These results support the potential of quercetin as a therapeutic agent for pressure ulcers (Yin et al. 2018). LPS administration provides protection against IR through the upregulation of Hsp27. Preconditioning-induced Hsp27 upregulation holds potential for the treatment of renal IR by reducing apoptosis (He et al. 2021).

Ten aquaporin subtypes (AQP1 through AQP11) were examined using RT-qPCR (real-time polymerase chain reaction). Organ-specific AQP expression profiles were generated and subjected to principal component analysis. Protein expression of AQP1, AQP2, AQP3, and AQP4 in rat kidneys was evaluated via immunohistochemistry. It was found that the expression of these aquaporins was significantly reduced in rat kidneys subjected to MCAO/reperfusion; the kidney

was identified as being at the highest risk of damage following CIR/I, and the downregulation of AQP2, AQP3, and AQP4 was implicated in CIR/I-induced acute kidney injury (Dai et al., 2024). Male BALB/c mice were injected with siRNA targeting SPARC (Secreted Protein Acidic and Rich in Cysteine) prior to 45 minutes of renal ischemia, followed by 72 hours of reperfusion; post-I/R analysis revealed increased levels of serum creatinine, blood urea nitrogen, tubular damage, tubulointerstitial fibrosis, and the expression of collagen I and TGF-β. Levels of the oxidative stress marker 4-hydroxy-2-nonenal and the inflammatory cytokines MCP-1 and TNF-α were also elevated. SPARC mRNA was found to be overexpressed and localized within damaged tubuloepithelial cells, co-localizing with ADAMTS1 expression. Treatment with SPARC siRNA attenuated renal dysfunction, histological damage, collagen accumulation, oxidative stress, and inflammation, while suppressing the I/R-induced increases in the expression of ADAMTS1 (A Disintegrin and Metalloproteinase with Thrombospondin motifs) and MMP-2/9. Consequently, SPARC may serve as a novel therapeutic target for acute kidney injury (Toba et al., 2025). Hepatic IR increased renal and pulmonary MMP2, MMP3, MMP9, and TIMP2 expression starting at 180 minutes. Silibinin treatment reduced MMP2, MMP3, and MMP9 levels while increasing TIMP2; these changes began 120 minutes after reperfusion and became significant in the kidney at 240 minutes. The effect of silibinin peaked 180 minutes after hepatic reperfusion. In conclusion, while hepatic IR led to renal and pulmonary injury, intravenous silibinin exerted a protective effect in the kidney (Kollaras et al. 2021).

1.2. Pathophysiology

Inflammation, mitochondrial damage, and extracellular matrix remodeling are key, mutually reinforcing components in the pathophysiology of renal I/R (Pan and Zhu 2026). Within this network, Notch signaling acts as one of the regulators of the injury response (Pan and Zhu 2026). Although basal Notch activity is limited in the adult kidney, the pathway is reactivated during acute and chronic injury. The Notch intracellular domain (NICD)—generated through sequential proteolytic cleavages following ligand-receptor binding—translocates to the nucleus and enhances target gene transcription (Mohammed and Rizij 2025). Reports of increased Delta-1, Hes-1, and Notch-2 expression in renal I/R models suggest that this axis may be associated with proliferative/repair processes and the tubular epithelial response (Mohammed and Rizij 2025).Increased mtROS production accelerates cell death through mitochondrial dysfunction and mPTP opening. The release of damage-associated molecules, such as mtDNA, activates inflammatory sensors, thereby propagating the injury (Pan and Zhu 2026). Regarding tubular function, alterations in AQP1 levels are considered an early indicator of impaired water transport capacity (Asvapromtada et al. 2018).In terms of matrix biology, an imbalance in MMP-9 and MMP-3 expression can play a decisive role in the transition from acute injury to fibrotic remodeling (Pan

and Zhu 2026; Dong et al. 2021). A reduction in fibrotic markers has been reported in AKI-to-CKD transition models following the modulation of PI3K/AKT and MAPK signaling pathways. A sequential mitochondrial damage cascade is described, progressing through ATP depletion, ionic imbalance, and Ca2+ overload during the ischemic phase, followed by an mtROS burst, collapse of ΔΨm, pathological mPTP opening, mtDNA damage, and mtDAMP release during the reperfusion phase (Pan and Zhu 2026). This cascade causes a sudden decline in the tubular cell's energy production capacity, leading to the failure of ion pumps and the subsequent disruption of membrane integrity, thereby exacerbating structural damage. The mtDAMP load generated upon reperfusion amplifies immune signals that fuel sterile inflammation, paving the way for local tissue damage to merge with a systemic inflammatory response. Consequently, while the intracellular energy crisis deepens, the process further perpetuates I/R injury by reinforcing programmed cell death pathways—driven by the impairment of mitochondrial quality control mechanisms (such as mitophagy, biogenesis, and dynamics) (Pan and Zhu 2026).In a study conducted by Mohammed and Rizij (2025), GSH and F2-isoprostane parameters were reported to assess the oxidative stress burden; a robust oxidative stress response—characterized by increased F2-isoprostane levels and decreased GSH—was demonstrated following I/R. The reduction in F2-isoprostane levels and the increase in GSH observed after silibinin pretreatment support the conclusion that antioxidant defense mechanisms partially recover and free radical-induced damage is mitigated (Mohammed and Rizij 2025). It has been shown that Notch-1/Jagged-1 mRNA expression levels increase following I/R and are significantly reduced by silibinin pretreatment, supporting the regulatory role of the Notch axis in the pathobiology of renal I/R (Mohammed and Rizij 2025). Compounds such as hesperidin, quercetin, naringenin, vitexin, and luteolin have been reported to reduce tissue damage through Nrf2/HO-1 activation, NF-κB suppression, and the preservation of mitochondrial function (Bagheri et al. 2023; Chen et al. 2025; Meng et al. 2020; Wei et al. 2025; Zhang et al. 2022).

It has been reported that tangeretin suppresses the MAOA/NF-κB axis and reduces inflammation while improving renal function and histopathological damage in a renal I/R model (Gao et al., 2025). Similarly, it has been shown that luteoloside alleviates renal I/R injury by suppressing ferroptosis through the modulation of the KEAP1-Nrf2 interaction (Qiu et al., 2026). Xu et al. (2025) demonstrated that apigenin upregulates Nrf2/HO-1 and tight junction proteins while suppressing the release of inflammatory cytokines, including IL-1β and IL-6, in an intestinal I/R model (Xu et al., 2025). Flavonoids can target common injury nodes—such as ferroptosis, inflammation, mitochondrial quality control, and oxidative stress—across various organ I/R models (Gao et al., 2025; L. Yan et al., 2025; Qiu et al., 2026; Xu et al., 2025). IL-1β and IL-6 are pro-inflammatory cytokines that serve as indicators of the inflammatory burden

(Danis et al., 2024). NF-κB is a key signaling node that regulates the transcription of numerous inflammatory genes (Tian et al., 2024). Nrf2 activates antioxidant response elements; it is also a transcription factor that maintains cellular redox balance (Wei et al., 2025; Zhang et al., 2022).

AQP1 is a functional membrane channel that regulates renal water transport, particularly in the proximal tubule (Asvapromtada et al., 2018). MMP-3 and MMP-9 are matrix metalloproteinases involved in extracellular matrix remodeling and tissue restructuring (Pan and Zhu 2026; Dong et al. 2021). Following I/R, TLR4/mtDAMP signaling enhances NF-κB activation, leading to elevated levels of IL-6 and IL-1β (Feng et al. 2024). Concurrently, as oxidative stress increases, activation of the Nrf2 axis is triggered as a protective, compensatory response (Wei et al. 2025). Furthermore, as tubular damage progresses, alterations in AQP1 patterns result in impaired water transport capacity (Asvapromtada et al. 2018). MMP-9 and MMP-3 serve as markers reflecting the fibrotic shift during the transition from acute inflammatory injury to chronic remodeling (Dong et al. 2021; Pan and Zhu 2026). Considering the functional dynamics within the renal I/R process, NF-κB acts as a regulatory hub for the initiation and maintenance of the inflammatory response (Pan and Zhu 2026). IL-1β and IL-6 are indicators that emerge following this activation, reflecting the severity of local and systemic inflammation (Danis et al. 2024). Nrf2 constitutes the primary defense axis for limiting oxidative damage and preserving tubular cell integrity (Wei et al. 2025; Zhang et al. 2022).

Gao et al. (2025) demonstrated that the target spectrum of flavonoids in renal I/R biology extends beyond the IL-1β, IL-6, NF-κB, and Nrf2/HO-1 axes, highlighting the significance of nodes such as MAOA/NF-κB. Qiu et al. (2026) reported that luteoloside suppresses ferroptosis by disrupting the KEAP1-Nrf2 interaction; this finding indicates that interpretations regarding Nrf2 should not be limited solely to antioxidant enzyme induction. Studies on non-renal I/R models have shown that the suppression of inflammatory cytokines, preservation of barrier and mitochondrial integrity, Nrf2 activation, and the promotion of mitophagy are recurring components of flavonoid action (Xu et al. 2025; Yan et al. 2025).

1.3 Effect of Flavonoids on IL-1β and IL-6 Levels in Renal Ischemia-Reperfusion

IL-6 stands out as a significant pro-inflammatory cytokine in ischemia-reperfusion (I/R) injury. In a study by Zhang et al. (2022), it was demonstrated that naringenin administration significantly reduced IL-6 levels in I/R renal tissue and an HK-2 H/R model, and that this reduction was associated with Nrf2/HO-1 activation. Yang et al. (2017) administered diosmetin in a mouse renal I/R model and examined Cr, BUN, histopathology, and inflammatory markers; they reported that IL-6 levels decreased compared to the injury group and that this

decrease paralleled functional and histological recovery. Hesperidin treatment in a rat I/R model showed that IL-6 was suppressed compared to the I/R group (Meng 2020). Peng et al. (2023) reported that flavonoids reduce the inflammatory burden in renal I/R by suppressing the rise in IL-6 across various experimental models. In an animal I/R model treated with quercetin, IL-6 was measured alongside tissue damage indicators and oxidative stress enzymes, and a decrease in IL-6 was reported in the treatment group (Bagheri et al. 2023). Danis et al. (2024) reported that the reduction in IL-6 observed in an experimental renal I/R model using naringin was consistent with improvements in other markers of injury. In a study conducted by Wei et al. (2025), the effect of luteolin on IL-6 was evaluated through a three-stage validation process: (i) network pharmacology analysis identified inflammation-related common targets by comparing luteolin-target clusters with renal I/R-target clusters, and pathway enrichment analysis highlighted the prominence of IL-6-associated inflammatory axes; (ii) molecular docking confirmed the binding affinity of luteolin with candidate proteins, supporting the likelihood of biological interaction; (iii) experimental validation utilized both a UIRI rat model and an HK-2 cell hypoxia/reoxygenation model, examining the IL-6 response in both cellular and tissue contexts. An inflammatory panel—including IL-6—was measured, alongside assessments of histopathology (H&E), renal function (Cr, BUN), apoptotic markers (Bax/Bcl-2, cleaved caspase-3), and oxidative stress indicators. It was reported that luteolin suppressed IL-6 levels and patterns of inflammatory/oxidative damage, providing a protective effect that could both reduce acute inflammation and mitigate the persistence of injury during the transition from AKI to CKD. The study, which employed both the UIRI rat model and the HK-2 cell hypoxia/reoxygenation model, reported that luteolin reduced IL-6 levels in conjunction with decreased oxidative stress and histological improvement (Wei et al., 2025).

In a study conducted by Yang et al. (2017) using a mouse renal I/R model, diosmetin was administered. IL-6 levels rose in the I/R group but decreased in the group treated with diosmetin. This reduction paralleled improvements in epithelial shedding, interstitial congestion, and tubular necrosis. In a study conducted by Güvenç et al. (2020), nobiletin was administered orally at a dose of 10 mg/kg/day for 7 days, followed by the induction of a renal I/R model via clamping of the left renal artery for 45 minutes and subsequent reperfusion for 3 hours. The study evaluated IL-6 and IL-1β levels in renal tissue. The results showed that the elevated IL-6 levels observed in the I/R group were significantly reduced in the group pre-treated with nobiletin, with values approaching control levels. It has been demonstrated that the increase in IL-6 and IL-1β is associated with the NF-κB target gene response activated by ischemia, and that nobiletin exerts an anti-inflammatory effect by suppressing this proinflammatory cytokine profile (Yang et al., 2017).Zhang et al. (2022) demonstrated that naringenin

significantly reduces IL-6 levels in both an *in vivo* renal I/R model and an HK-2 hypoxia/reoxygenation model.Silibinin is a natural flavonolignan isolated from *Silybum marianum* seeds. In a study by Mohammed MH and Rizij FJ (2025), ischemia was induced for 30 minutes followed by 2 hours of reperfusion using bilateral renal pedicle clamping. Silibinin was administered intraperitoneally (i.p.) at a dose of 60 mg/kg one hour prior to the induction of ischemia. Existing evidence regarding its renoprotective effects indicates that silibinin can mitigate kidney damage by suppressing inflammation and oxidative stress. It also appears to regulate cell death and fibrotic remodeling processes via the PI3K/AKT-MAPK axes (Mohammed and Rizij, 2025).

The effect of silibinin on renal I/R injury was evaluated at both in vivo and in vitro levels. Models representing AKI (24 hours) and the AKI-to-CKD transition (14 days) were established in 10-week-old male mice; sham and I/R groups were compared following treatment with either silibinin (100 mg/kg, oral gavage, 7 days preoperatively) or a vehicle. In the surgical model, ischemia was induced by clamping the left renal pedicle for 30 minutes following right nephrectomy. At the cellular level, silibinin was administered prior to HK-2 cell models of hypoxia/reoxygenation and TGF-β1-induced fibrosis, and assessments were made regarding MAPK pathways, inflammation (TNF-α, IL-1β, IL-6), PI3K/AKT signaling, and fibrosis-related proteins. The findings indicate that silibinin improves renal function, suppresses oxidative damage exacerbated by I/R, and provides a protective effect that may limit the progression from AKI to CKD. It was demonstrated that silibinin delays the transition from AKI to CKD by enhancing PI3K/AKT phosphorylation and reducing oxidative stress, inflammation, and fibrosis (Dong et al., 2025).

1.4. Renal Ischemia-Reperfusion and NF-κB

In the context of I/R, NF-κB serves as a central signaling node that regulates proinflammatory cytokines and bridges cellular stress, mitochondrial damage, and innate immune sensing systems (Dong et al. 2025; Pan and Zhu 2026). ATP depletion during ischemia, Ca2+ overload, and oxidative stress—which becomes pronounced upon reperfusion—promote NF-κB activation through IκB degradation and p65 nuclear translocation. Consequently, the expression of mediators such as IL-1β and IL-6 increases (Güvenç et al. 2020). Pan and Zhu (2026) demonstrate that mtDNA and other mtDAMPs released from mitochondria enhance the NF-κB-associated inflammatory response by activating sensors such as cGAS-STING, NLRP3, and TLR9. Dong et al. (2025) show that strengthening the PI3K/AKT axis and suppressing MAPK phosphorylation can reduce the NF-κB-associated cytokine burden, suggesting that this pathway may serve as a therapeutic target—a common final pathway—in renal I/R injury. Therefore, the NF-κB axis acts as a central molecular node for both the initiation of the early inflammatory response and the progression of

injury in renal I/R (Dong et al. 2025; Güvenç et al. 2020; Pan and Zhu 2026). NF-κB suppression is a central mechanism underlying the shared anti-inflammatory effects of flavonoids such as hesperidin, naringenin, and quercetin (Peng et al. 2023). Oxidative stress and inflammation are two fundamental components in the pathogenesis and progression of acute kidney injury (Güvenç et al., 2020). NF-κB and Nrf2 are among the key transcription factors that regulate inflammatory and oxidative stress responses, respectively. According to a study by Güler et al. (2020), the hypoxia and reoxygenation occurring during I/R enhance inflammatory signaling. It has been reported that the ischemia process upregulates the expression of IL-1β, IL-6, and TNF-α, which are among the NF-κB target genes. The significant reduction of these three pro-inflammatory cytokines following nobiletin pretreatment in the same study indicates that nobiletin indirectly suppresses the NF-κB-associated inflammatory response in I/R injury. In a study conducted by Meng et al. (2020), it was demonstrated that hesperidin suppresses NF-κB activity and reduces inflammatory damage. In the study, following the induction of renal I/R injury in rats, hesperidin was administered intravenously at doses of 10 or 20 mg/kg for 14 days, starting one day after surgery. At the molecular level, hesperidin administration was shown to decrease the expression of p-IκBα and NF-κB p65. Histopathological examination also revealed a reduction in the severity of tubular/parenchymal damage. These findings support the conclusion that hesperidin's protective effect against renal I/R injury is mediated by the suppression of the TLR-4/NF-κB/iNOS signaling axis (Meng et al., 2020). In a study by He et al. (2021), a nanoparticle form of apigenin loaded onto a biodegradable polymer carrier was used to enhance its bioavailability. They analyzed the NF-κB-related mechanism at both in vivo and in vitro levels. In the in vivo setup, the left renal artery was clamped for 30 minutes followed by 24 hours of reperfusion in 36 mice after a right nephrectomy. Apigenin was administered intraperitoneally at doses of 5, 10, and 20 mg/kg, 24 hours prior to surgery. The results demonstrated that apigenin restored miR-140-5p levels (which had been reduced by H/R), attenuated H/R-induced inflammatory cell injury, and suppressed both the translocation of NF-κB p65 from the cytoplasm to the nucleus and the activation of the pathway. Luciferase reporter assays revealed that miR-140-5p directly negatively regulates CXCL12, and that an increase in CXCL12 could attenuate apigenin's effect on NF-κB inactivation. Apigenin administration reduced the release of pro-inflammatory cytokines and the upregulation of CXCL12, while increasing miR-140-5p levels. Apigenin exerts a renoprotective effect in renal I/R injury by inhibiting NF-κB pathway activation through the upregulation of miR-140-5p and the suppression of CXCL12 (He et al., 2021). Bagheri et al. (2023) demonstrated the suppression of the NF-κB-related pro-inflammatory profile in a quercetin model. They showed that quercetin attenuates NF-κB activation in renal I/R injury and that this anti-inflammatory effect is confirmed at the protein expression level (Bagheri et al., 2023). Li et al. (2024) demonstrated that

increased NF-κB activity is associated with tubular injury and functional impairment. In their study, Karacaer et al. (2023) employed a multi-endpoint model to evaluate the inflammatory response associated with renal I/R in both kidney and lung tissues. Compared to the control group, NF-κB immunoreactivity and oxidative stress markers were significantly elevated in the IRI group. Conversely, groups treated with hesperidin exhibited a significant reduction in oxidative load and NF-κB staining intensity compared to the IRI group. These findings indicate that hesperidin suppresses NF-κB-mediated inflammatory activation in parallel with oxidative stress parameters during renal I/R and exerts a renoprotective effect in a dose-dependent manner. Pan and Zhu (2026) investigated mitochondrial DNA release in renal I/R.

1.5. Renal Ischemia-Reperfusion and Nrf2

Nrf2 is one of the key regulators of the oxidative stress response in renal I/R. It acts as a transcriptional protective factor that influences not only classical antioxidant defense but also the severity of inflammation, mitochondrial integrity, and susceptibility to ferroptosis (Pan and Zhu 2026, Qiu et al. 2026). Nrf2 activation strengthens the antioxidant defense network, particularly HO-1. It reduces lipid peroxidation and slows the progression of damage in tubular cells (Wei et al. 2025, Pan and Zhu 2026). Studies have shown that Nrf2 not only limits acute oxidative damage but can also influence the transition from AKI to CKD by weakening the pathological links between inflammation, apoptosis, inflammation severity, and fibrosis (Wei et al. 2025, Qiu et al. 2026). Therefore, Nrf2 plays a significant role in explaining the protective effects of flavonoids against renal I/R injury (Pan and Zhu 2026). Shokeir et al. (2015) demonstrated the effect of Nrf2 activation in limiting renal I/R injury. Nrf2 is not merely a transcription factor that increases antioxidant enzyme expression; it also interacts with networks governing mitochondrial biogenesis and energy homeostasis, such as those involving PGC-1α. Preserving the Nrf2 axis enhances ROS detoxification and limits the accumulation of damaged mitochondria. When the Nrf2 response is insufficient, oxidative burden and inflammatory activity are prolonged. Nrf2/HO-1 activation proceeds in parallel with functional recovery (Pan and Zhu 2026). In a study by Jo et al. (2023), a bilateral renal I/R model in aged (20-month-old) and young (2-month-old) mice, Nrf2 knockout (Nrf2−/−) mice, and senescent RPTEC cells exposed to H/R were evaluated together. Renal injury following I/R is more severe in aged models and in those with Nrf2 deficiency. Administration of the Nrf2 activator CDDO-Me partially reversed this condition, whereas Nrf2 siRNA-mediated silencing in RPTECs exacerbated H/R-induced injury. These findings indicate that Nrf2 is a key protective factor determining not only the acute antioxidant response but also mitochondrial recovery and tissue repair following I/R (Jo et al. 2023).

1.5.1 Effect of Flavonoids on Nrf2 Levels in Renal Ischemia-Reperfusion

The Nrf2/HO-1 antioxidant axis is a recurring key pathway involved in the renoprotective effects of flavonoids, and its activation coincides with improvements in oxidative stress markers (Peng et al. 2023). Early-phase Nrf2 hyperactivation has been shown to reduce the progression of tubular injury (Nezu et al. 2017).In renal I/R injury, ER stress, pyroptosis, and apoptosis are interrelated primary injury axes, and naringenin suppresses this axis through Nrf2/HO-1 activation (Zhang et al. 2022).In the study by Güvenç et al. (2020), Nrf2 was not directly measured; However, it has been emphasized that inflammation and oxidative stress are among the primary factors driving the progression of acute kidney injury, and that Nrf2 is a key transcription factor regulating the cellular response to oxidative stress. In the same study, the observation of improvements in antioxidant parameters—such as GSH, GSH-Px, and catalase—and a reduction in MDA levels following nobiletin administration suggests that nobiletin may exert a protective effect aligned with the Nrf2-mediated antioxidant defense axis. Nevertheless, this conclusion relies on an indirect assessment based on oxidative stress markers rather than direct Nrf2 measurement.It has been confirmed that naringenin increases Nrf2 and HO-1 expression at both the cellular level and within renal tissue. The study tested the contribution of the Nrf2 pathway using ML385 (an Nrf2 inhibitor) and demonstrated that ML385 attenuated the antioxidant/anti-inflammatory protective effects of naringenin (Zhang et al., 2022).In the study by Wei et al. (2025), luteolin-target and renal I/R-target clusters were identified via systematic network pharmacology, and interaction network and pathway enrichment analyses were performed based on shared targets; (ii) the binding affinity of luteolin to candidate target proteins was confirmed through molecular docking; and (iii) molecular, biochemical, and histopathological validation was conducted using an *in vivo* UIRI rat model representing the transition from AKI to CKD (Wei et al., 2025). Molecular docking results further supported luteolin's potential to bind to these shared targets, thereby reinforcing the concept of multi-target/multi-pathway interactions. This finding supports the view that luteolin exerts its effects through multiple biological pathways rather than a single axis. In the in vivo validation phase, using a rat model of unilateral ischemia-reperfusion (UIRI) that simulates the transition from AKI to CKD, it was demonstrated that luteolin administration reduced renal injury and improved markers of oxidative stress, inflammation, and fibrotic remodeling. It was determined that luteolin mitigates I/R-induced oxidative damage by suppressing lipid peroxidation in renal tissue and that this effect is associated with the activation of the Nrf2/HO-1 antioxidant axis (Wei et al., 2025).

The most significant finding regarding Nrf2 is that luteolin [strengthens] the Nrf2/HO-1 antioxidant axis. The study also found that AST treatment resulted in a reduction of brush border loss, tubular degeneration, and intratubular debris at

the histopathological level; an attenuation of the rise in serum creatinine and BUN; a decrease in ROS/MDA levels (oxidative stress) and an increase in SOD activity (antioxidant defense); and the suppression of the NLRP3, ASC, cleaved-caspase-1, GSDMD-N, and IL-1β axis (inflammatory cell death). Regarding the dose-response pattern, medium-to-high doses of AST provided more pronounced protection. A similar effect, intensifying with increased concentration, was also observed in the cell model. In the mechanism validation phase, the observation that Sirt1 blockade weakened the Nrf2/HO-1 response and led to a resurgence of oxidative/pyroptotic damage demonstrated that AST exerts its primary effect by activating the Nrf2 antioxidant program via SIRT1. Consequently, it was determined that Nrf2 activation is not merely a concomitant biochemical finding; rather, it is a central mechanism that aligns with the control of oxidative stress (ROS, MDA, SOD), functional recovery (Cr, BUN), morphological preservation (histology), and the suppression of inflammatory cell death (NLRP3-IL-1β axis) (Huang et al., 2025). In a study by Zhang et al. (2022), it was demonstrated that naringenin reduces inflammatory damage and oxidative stress in a renal I/R model by activating the Nrf2/HO-1 axis. In a mouse model involving 30 minutes of ischemia followed by 24 hours of reperfusion, naringenin administration led to increased Nrf2 and HO-1 protein expression in renal tissue. Naringenin has also demonstrated an Nrf2-mediated protective effect in the HK-2 hypoxia/reoxygenation (H/R) model. This protective effect is attenuated upon administration of the Nrf2 inhibitor ML385, indicating that Nrf2/HO-1 activation plays a direct role in the renoprotective effect of naringenin. Naringenin enhances Nrf2 signaling, thereby boosting the antioxidant response and improving renal function markers and histological damage. This effect has also been confirmed in HK-2 cells under H/R conditions, where Nrf2 inhibition using brusatol was shown to reduce the protective efficacy (Zhang et al., 2022).

1.6. Effect of Flavonoids on IL-1β Levels in Renal Ischemia-Reperfusion

In a study conducted by Zhang et al. (2022), it was found that naringenin administration significantly reduced IL-1β levels in both I/R kidney tissue and the HK-2 H/R model. This reduction paralleled Nrf2/HO-1 activation. The attenuation of this effect by the Nrf2 inhibitor ML385 demonstrated that the reduction in IL-1β occurred via an Nrf2-dependent mechanism (Zhang et al., 2022). In the HK-2 H/R model, naringenin was administered at a concentration of 200 μM, while in the C57BL/6 mouse I/R model, it was administered via oral gavage at 50 mg/kg/day for 3 days. Improvements in renal function and a reduction in tissue damage were observed, alongside a decrease in IL-1β levels.

Nobiletin has been reported to selectively reduce COX-2 gene and protein expression in various experimental systems. It suppresses IL-1β production in mouse macrophage cells (Güvenç et al., 2020). Therefore, nobiletin is considered

a candidate flavonoid capable of suppressing the proinflammatory cytokine response in renal I/R models (Güvenç et al., 2020).

In the study by Güvenç et al. (2020), rats were administered nobiletin orally at 10 mg/kg/day for 7 days, followed by the establishment of an I/R model involving 45 minutes of ischemia and 3 hours of reperfusion in the left kidney. Nobiletin administration significantly lowered the IL-1β levels that had been elevated by I/R; this reduction brought levels close to those of the control group. Biochemical and histological improvements also demonstrated a reduction in tissue inflammation. Ischemic conditions increased the expression of IL-1β, IL-6, and TNF-α genes—targets of the NF-κB pathway—whereas nobiletin exerted an anti-inflammatory effect by suppressing this cytokine elevation. Regarding IL-1β, nobiletin can attenuate the cytokine response that exacerbates early inflammatory activation and tissue damage during I/R injury (Güvenç et al., 2020). The fact that the decrease in IL-1β levels coincided with a reduction in histological damage scores indicates that nobiletin does not merely induce biochemical changes but also mitigates the severity of tubular and interstitial inflammatory damage (Güvenç et al., 2020). Wang et al. (2024) demonstrated that, in an I/R-AKI model, the reduction in IL-1β paralleled a decrease in macrophage infiltration and the MIF axis.

In a study conducted by Mohammed MH and Rizij FJ (2025), 28 male Wistar albino rats (weighing 250–300 g) were divided into four groups—sham, I/R, DMSO, and I/R+silibinin—with seven rats per group. The model was established using bilateral renal pedicle clamping involving 30 minutes of ischemia followed by 2 hours of reperfusion; silibinin (60 mg/kg, i.p.) was administered one hour prior to the induction of ischemia. IL-1β levels increased significantly compared to the sham group. In the I/R+silibinin group, there was a significant decrease compared to the I/R group; however, following treatment, the values did not fully return to sham levels and remained elevated relative to the sham group.

60 mg/kg i.p. administered 1 hour before ischemia Silibinin administration significantly reduced IL-1β levels; however, values in the treatment group remained significantly higher than those in the sham group. Studies by Mohammed MH and Rizij FJ (2025) and Dong et al. (2025) found that IL-1β levels, which increased following I/R injury, decreased significantly after treatment. The increase in IL-1β in the I/R group compared to the sham group was statistically significant. In the I/R+silibinin group, IL-1β levels decreased relative to the I/R group. It was noted that post-treatment IL-1β values did not fully return to sham levels (Mohammed and Rizij, 2025). Similarly, the study by Dong et al. (2025) demonstrated that the IL-1β response, elevated by I/R, was suppressed at both the protein and mRNA levels following silibinin administration. In a study by Zhang et al. (2022), a renal I/R model was established in mice involving 30 minutes of ischemia followed by 24 hours of

reperfusion; animals were assigned to Sham I/R, I/R+NS, I/R+NRG, and I/R+4-PBA groups, receiving oral naringenin (NRG) and intraperitoneal (i.p.) 4-PBA, respectively. The pyroptosis-mitigating effect of NRG was confirmed by reduced protein expression of NLRP3, ASC, caspase-1 p10, GSDMD-N, caspase-11, and mature IL-1β in the NRG+I/R group. Administration of the ER stress inhibitor 4-PBA markedly suppressed the activation of pyroptosis markers and was associated with a reduction in IL-1β levels (Zhang et al., 2022).

Conclusion

Renal ischemia-reperfusion (I/R) injury is a major cause of acute kidney injury and represents a complex pathophysiological process involving the interplay of energy depletion, oxidative stress, inflammation, mitochondrial dysfunction, and programmed cell death mechanisms. Cellular damage, initiated by ATP depletion and the disruption of ionic balance during the ischemic phase, is further exacerbated following reperfusion by increased reactive oxygen species (ROS) production and the activation of inflammatory responses. In particular, NF-κB-mediated pro-inflammatory cytokine production and alterations in the Nrf2/HO-1 axis play decisive roles in the progression of renal tissue damage. Studies examining inflammatory processes and the role of flavonoids in renal ischemia demonstrate that inflammatory markers such as IL-1β and IL-6 serve as significant biomarkers in renal I/R injury and correlate closely with the severity of the damage.

Experimental findings in the literature indicate that flavonoids—including quercetin, naringenin, hesperidin, luteolin, nobiletin, fisetin, astragalin, and silibinin—exert significant protective effects against renal I/R injury. It is reported that the antioxidant, anti-inflammatory, anti-apoptotic, and anti-fibrotic properties of these compounds enable the reduction of oxidative stress, suppression of inflammatory cytokine production, preservation of mitochondrial function, and improvement of renal function. Key mechanisms underlying the protective actions of flavonoids include the suppression of NF-κB activity, activation of the Nrf2/HO-1 pathway, and the mitigation of damage mechanisms such as ferroptosis and pyroptosis. In conclusion, flavonoids stand out as potential pharmacological agents for the prevention and treatment of renal I/R injury. However, the vast majority of existing data is based on experimental animal and cell culture studies. Therefore, large-scale clinical trials are needed to determine the dosage, timing of administration, bioavailability, and safety profiles of various flavonoids. A more detailed elucidation of the molecular pathways targeted by flavonoids could contribute to the development of novel protective and therapeutic strategies for renal I/R injury in the future.

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CHAPTER 12

Microplastics in Aquatic Ecosystems: Occurrence, Impacts on Fish, and Human Health Risks

***Belgin Siriken*[1]**

[1] Prof. Dr. Department of Aquatic Animal Diseases, Faculty of Veterinary Medicine, Ondokuz Mayıs University, Samsun, Türkiye, ORCID:0000-0002-5793-1792

1. INTRODUCTION

Plastics are synthetic polymeric materials characterized by their versatility, durability, lightweight structure, chemical resistance, and relatively low production cost, making them indispensable in a wide range of industrial and consumer applications (Andrady & Neal, 2009; Jadhav et al., 2023). Owing to these advantageous properties, global plastic production has increased continuously since the 1950s, reaching 430.9 million metric tons (Mt) in 2024. Europe accounted for 54.6 Mt of global plastics production in the same year (PlasticsEurope, 2025). However, the extensive production and widespread use of plastics, particularly in short-lived applications such as packaging, have resulted in the accumulation of plastic waste in terrestrial and aquatic environments, where larger plastic debris gradually fragments into micro- and nanoplastics. These particles are widely distributed throughout aquatic ecosystems and can be readily ingested by aquatic organisms, including fish and shellfish, thereby facilitating their transfer through the food chain. Consequently, the consumption of seafood contaminated with micro- and nanoplastics has emerged as a potential route of human exposure, raising growing concerns regarding food safety and public health (Hamlin et al., 2015; Muncke et al., 2020). The potential health effects of micro- and nanoplastics largely depend on their physicochemical characteristics and the chemical additives they contain. These materials have been associated with carcinogenic potential, neurotoxicity, and endocrine-disrupting effects (Karbalaei et al., 2018; Rist et al., 2018). Furthermore, nanoplastics have been reported to penetrate biological membranes and cross physiological barriers, including the placental and blood–brain barriers, thereby reaching critical organs and posing additional risks to human health (Bouwmeester et al., 2015; Rist et al., 2018).

1.1 Sources and Environmental Distribution of Plastics

Plastics are synthetic polymeric materials composed of long-chain macromolecules formed through the polymerization of monomers. Owing to their durability, lightweight structure, water resistance, low production cost, and ease of processing, plastics have become indispensable materials in numerous industrial and consumer applications. Plastics are classified into seven major categories: polyethylene terephthalate (PET), high-density polyethylene (HDPE), polyvinyl chloride (PVC), low-density polyethylene (LDPE), polypropylene (PP), polystyrene (PS), and other plastics (Andrady & Neal, 2009). Depending on their response to heat, plastics are generally classified as thermoplastics, thermosets, and thermoplastic elastomers, while their molecular structure further categorizes them as amorphous or semi-crystalline polymers (MMO, 2018).

Polyethylene (PE) is the most widely produced polymer worldwide, followed by polypropylene (PP), polyvinyl chloride (PVC), polyethylene terephthalate (PET), and polystyrene (PS), which are extensively used in packaging, construction, automotive, electrical, and consumer products (Plastics Europe AISBL, 2025). Packaging remains the largest application sector for plastics because of its extensive use and short service life, making it one of the major

contributors to plastic waste entering the environment. Improper disposal and inadequate waste management allow plastic debris to accumulate in terrestrial and aquatic ecosystems, where environmental weathering, ultraviolet radiation, mechanical abrasion, and biological processes progressively fragment larger plastic items into microplastics and, ultimately, nanoplastics

1.2. Plastic Pollution in the Aquatic Environment

The excessive production, widespread use, and improper disposal of plastics have resulted in severe plastic pollution across terrestrial, freshwater, and marine ecosystems (Phuong et al., 2016; Tokaç, 2018). In particular, the increasing consumption of short-lived single-use plastic products, together with inadequate waste management practices, has substantially intensified environmental contamination (Lusher et al., 2017; Tokaç, 2018). Plastic waste originates from a variety of sources, including municipal discharges, industrial activities, fisheries, shipping, and other anthropogenic activities. Mismanaged plastic waste is transported through rivers, wastewater, and coastal runoff into marine environments, where it accumulates and persists for long periods (Barboza et al., 2018; Karthik et al., 2018).

According to the International Union for Conservation of Nature (IUCN, 2022), plastics account for approximately 80% of all marine litter (Carney and Eggert, 2019), and nearly 14 million tonnes of plastic waste enter the oceans annually. Recent estimates by the Organisation for Economic Co-operation and Development (OECD, 2022) indicate that approximately 1.4 million tonnes of plastic were transported to marine environments via rivers in 2020, with river systems representing the dominant pathway of land-based plastic pollution. The continued increase in plastic production, coupled with inefficient waste management, has accelerated the accumulation of plastic debris in marine ecosystems, posing a growing threat to aquatic biodiversity and ecosystem functioning.

Plastic debris has been detected throughout the marine environment, from surface waters to deep-sea sediments, and can be transported over long distances by ocean currents (Eriksen et al., 2014; Lusher et al., 2017). Modeling studies predict that, without significant improvements in global waste management systems, the cumulative amount of plastic waste in terrestrial, freshwater, marine, and atmospheric environments could reach 13.2 billion tonnes by 2050 (Silva et al., 2020). Because plastics degrade extremely slowly under natural environmental conditions, they accumulate continuously in ecosystems. Furthermore, fragmentation is a prolonged process; for example, the degradation of a 1-mm plastic particle into nanoscale fragments in marine environments has been estimated to require approximately 320 years (Jovanović, 2017). Consequently, plastic pollution represents a persistent and long-term environmental challenge, contributing to the continuous generation of micro- and nanoplastics that threaten aquatic organisms and, ultimately, human health through the food chain.

1.3. Microplastics and Nanoplastics

Microplastics (MPs) are generally defined as synthetic polymer particles smaller than 5 mm that are highly persistent and insoluble in water (Frias & Nash, 2019). Despite this general definition, several definitions of MPs have been proposed in the literature. For instance, 'Synthetic solid particles or polymeric matrices that are insoluble in water, originating from primary or secondary production, with different shapes (regular or irregular) and sizes ranging from 1 μm to 5 mm (maximum dimensions less than 5 mm) are called microplastics' (Frias and Nash, 2018); another definition is 'Plastics with particles measured between 1 μm and 100 nm and sub-micron are microplastics' (ISO, 2020); or according to the European Food Safety Authority (EFSA, 2016), microplastics are plastic particles ranging from approximately 0.1 μm to 5 mm, whereas particles smaller than 100 nm are generally considered nanoplastics (EFSA, 2016). The last definition, plastics smaller than 100 nm are considered microplastics (GESAMP, 2016; ISO, 2020; Bauwmeester et al., 2015). MPs comprise a very heterogeneous collection of particles that differ in size, shape, and chemical composition, among other properties (Hidalgo-Ruz et al., 2012; Andrady, 2017).

Owing to their small size, widespread distribution, and resistance to degradation, microplastics have become ubiquitous contaminants in terrestrial, freshwater, and marine environments. Nanoplastics (NPs), commonly defined as plastic particles smaller than 1 μm, represent the smallest fraction of plastic debris and exhibit distinct physicochemical properties, including a higher surface-area-to-volume ratio and greater biological reactivity than larger plastic particles (da Costa et al., 2016; ISO, 2020).

Based on their origin, microplastics are classified as either primary or secondary. Primary microplastics are intentionally manufactured at microscopic sizes for use in products such as cosmetics, personal care products, industrial abrasives, and biomedical applications. In contrast, secondary microplastics are generated through the fragmentation of larger plastic items through environmental weathering processes, including ultraviolet (UV) radiation, mechanical abrasion, oxidation, and biological degradation (GESAMP, 2015; Yurtsever, 2018). Inadequate waste management, wastewater discharges, textile fibers, tire wear particles, fishing activities, and plastic packaging are recognized as major contributors to microplastic contamination in aquatic environments.

As environmental degradation progresses, both macroplastics and microplastics continue to fragment into progressively smaller particles, ultimately forming nanoplastics.

Compared with microplastics, nanoplastics exhibit greater environmental mobility and can more readily penetrate biological membranes owing to their extremely small size. Consequently, micro- and nanoplastics have attracted increasing attention due to their persistence in aquatic ecosystems, their potential for bioaccumulation and trophic transfer, and their possible adverse effects on aquatic organisms and human health.

1.4. Environmental Fate, Trophic Transfer, and Biological Effects of Micro- and Nanoplastics

Microplastics are highly persistent contaminants that resist degradation and therefore accumulate in terrestrial, freshwater, and marine ecosystems. They have been detected in surface waters, sediments, drinking water, and even the atmosphere, demonstrating their widespread environmental distribution (Cózar et al., 2017; Frias & Nash, 2019; Koelmans et al., 2019). Their environmental fate is largely influenced by polymer density. Low-density polymers, such as polyethylene (PE) and polypropylene (PP), tend to remain suspended or float near the water surface, whereas denser polymers, including polyvinyl chloride (PVC), polystyrene (PS), polyamide (PA), and polyethylene terephthalate (PET), are more likely to settle in bottom sediments (Guo & Wang, 2019). Consequently, microplastics are distributed throughout both pelagic and benthic zones of coastal and marine systems, where they may persist for decades or even centuries due to their slow degradation rates (Jovanović, 2017). Salinity is one of the main factors affecting the chemical degradation of plastic. Therefore, coastal and marine systems with salinity ranging from about 0.5 to 35/00 (ppt: parts per thousand) are highly susceptible to microplastic formation (Thushari and Senevirathna, 2020).

MPs are highly persistent in the environment and therefore accumulate at increasing rates in different marine ecosystems. They are also very abundant in freshwater and drinking water, and it has been reported that fibers, films, foams, and pellets are the most common forms of microplastics found in surface water samples (Koelmans et al., 2019). Since MPs can easily circulate in water and air, they are likely to enter products from production processes or from the air. The way plastics reach the seas and oceans has remained at this level for years.

Beyond their physical persistence, microplastics also act as vectors for a wide range of environmental contaminants. Their large specific surface area enables the adsorption of persistent organic pollutants (POPs), polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), heavy metals, and plastic additives such as bisphenol A (BPA) and phthalates, facilitating the transport of these contaminants within aquatic ecosystems (Bouwmeester et al., 2015; GESAMP, 2016; Hirai et al., 2011; Lithner et al., 2011; Rios et al., 2010). This combination of physical particles and associated chemicals increases the ecological risks posed by plastic pollution.

1.4. Microplastics in Fish and Other Aquatic Organisms

Microplastics are readily ingested by a wide range of aquatic organisms, including zooplankton, benthic invertebrates, fish, marine mammals, and seabirds, allowing their transfer through aquatic food webs (Kumar et al., 2018; Wright et al., 2013). Fish are exposed to microplastics directly from contaminated water and sediments or indirectly through the consumption of contaminated prey. Although some particles are excreted, others may accumulate in the gastrointestinal tract and, particularly for smaller particles, translocate to other tissues, inducing oxidative stress, inflammation, and cellular damage (Auta et al.,

2017; Roch et al., 2020; Rubin et al., 2021). Compared with microplastics, nanoplastics exhibit greater mobility. They are more capable of crossing biological membranes, thereby increasing their potential for bioaccumulation, trophic transfer, and adverse effects on aquatic organisms and human health (Bouwmeester et al., 2015; da Costa et al., 2016).

Aquatic organisms readily ingest MPs because they closely resemble natural food particles in size, shape, and appearance. Consequently, exposure occurs across all trophic levels, from plankton and benthic invertebrates to commercially important fish species. Ingestion of microplastics has been associated with reduced feeding efficiency, impaired nutrient absorption, gastrointestinal obstruction, oxidative stress, inflammation, metabolic disturbances, immune dysfunction, and reduced growth and reproductive performance (Kershaw, 2016; Rist et al., 2018; Güven et al., 2017). Experimental studies have further demonstrated alterations in antioxidant enzyme activities, lipid metabolism, and energy homeostasis following chronic exposure to microplastics (Capó et al., 2021; Lu et al., 2016).

The toxicity of MPs is strongly influenced by particle size, polymer composition, and associated chemical contaminants. Larger particles generally remain within the gastrointestinal tract, whereas smaller MPs, particularly nanoplastics, can cross biological barriers and reach internal organs (Bouwmeester et al., 2015; EFSA, 2016). In addition, Mps act as carriers of persistent organic pollutants, heavy metals, endocrine-disrupting compounds, and plastic additives such as bisphenol A and phthalates, thereby enhancing their bioavailability and potential toxicity to aquatic organisms (Lithner et al., 2011; Rochman et al., 2013; Teuten et al., 2009). These combined physical and chemical effects may induce histopathological alterations, endocrine disruption, oxidative damage, and chronic inflammatory responses in exposed organisms.

Fish are exposed to microplastics through contaminated water, sediments, and prey organisms. Most ingested particles accumulate temporarily within the gastrointestinal tract and are subsequently excreted, although translocation to the liver, gills, and other tissues has also been reported, particularly for smaller particles (Grigorakis et al., 2017; Kıdeyş et al., 2018). The extent of tissue accumulation depends on particle size, polymer type, exposure duration, and feeding ecology. Although several experimental studies suggest that long-term accumulation in edible muscle tissue is relatively limited, continuous environmental exposure may increase the likelihood of tissue translocation and associated physiological effects.

Field studies conducted in Turkish marine waters consistently demonstrate the widespread occurrence of Mps in commercially important fish species from the Mediterranean, Aegean, Marmara, and Black Seas. Fibers are the predominant particle type, followed by fragments and films, while polyethylene and polypropylene are the most frequently identified polymers (Güven et al., 2017; Kıdeyş et al., 2018). Similar findings have been reported worldwide, indicating

that microplastic contamination has become a global concern affecting both marine and freshwater ecosystems.

Thousands of species from living groups such as seagrasses, phytoplankton, zooplankton, fish, benthic organisms, sea turtles, marine mammals, and seabirds that live in the aquatic ecosystem interact with the physical and chemical properties of the seas, forming the marine ecosystem. All organisms in the aquatic environment are connected through a food web, forming the marine ecosystem. Zooplankton are thought to absorb MPs as they obtain nutrients from the environment by consuming phytoplankton and smaller organisms. Benthic invertebrate organisms living in the marine ecosystem constitute the third link in the marine food chain after phytoplanktonic and zooplanktonic organisms. Benthic organisms are the most affected by plastic pollution in an environment because they usually live in or on sediment and move slowly (METU, 2017). In a laboratory study, it was found that ingested plastic particles, due to their small size, are usually found in the digestive tracts of living organisms. In addition, if very small nanoplastics are attached to algae in the sea, they are transported to fish through the food chain with zooplankton (Bouwmeester et al., 2015). MPs also pose an invisible hazard by entering the food chain and persisting in nature. The size of Mps fragments is similar to the size of feeding materials such as plankton and suspended particles (Wright et al., 2013). This characteristic allows invertebrates to ingest these synthetic microparticles. Benthic organisms and suspension feeders also feed on MPs from bottom sediments and polluted waters. Non-selective feeders collect and ingest all particles within a similar size range without separation via filter feeding or sediment feeding (Moore 2008; Browne et al. 2007). Ingestion of MPs by invertebrates depends on several factors, including feeding mechanism, type, shape, and quantity of the plastic material. Ward and Shumway (2004) reported that polystyrene MPs are highly susceptible to ingestion by filter-feeding bivalves. MPs in the marine environment are inadvertently consumed as bait by many marine organisms due to their immobility and color, and thus enter the food chain (Rist et al., 2018; Smith et al., 2018; Bouwmeester et al., 2015). When MPs are consumed by living organisms, they cause blockages in the digestive organs and reduce nutrient uptake (Rist et al., 2018; Kershaw, 2016). It has been reported that the growth rate is reduced in fish with high levels of microplastic particles in their diet (Naidoo and Glassom 2019), and a whale shark in Thailand probably died due to a single plastic straw found in its digestive tract (Haetrakul et al. 2009). Today, it is estimated that 90% of seabirds and 52% of sea turtles ingest plastic (Wilcox et al. 2015; Schuyler et al. 2015). Some study results have shown that food intake is altered or reduced, growth, immune system, fertility and reproduction, as well as cell function and behavior are altered in affected species, depending on the type and amount of plastic exposed (Dickerman and Goelet, 1987; Macedo et al. 2011; de Stephanis et al. 2013; Byrd et al. 2014; Prokić et al. 2019). In a 2017 study by Swedish researchers, it was found that freshwater sea bass larvae exposed to high levels of polystyrene in the sea preferred microplastics over natural foods. Researchers investigating the effects of MPs on fish life found that when bass larvae were exposed to polystyrene in water tanks, the growth rate of

fish larvae decreased by 81% when they consumed microplastics instead of food (Cebeci, 2017). According to recent studies, MPs have been shown to accumulate in the gastrointestinal tract of aquatic animals (Li et al., 2022; Ugwu et al., 2021), and MPs have been reported to increase catalase, glutathione reductase, glutathione-s-transferase, and induce antioxidant defense in the liver of fish (Sparus aurata) (Capo et al., 2021). Inhalation exposure to polystyrene micro (nano) plastics has recently been shown to cause inflammation in the lungs of rats (Lim et al., 2021). Furthermore, in a recent study, the first evidence of MPs (polypropylene) in the human placenta was reported (Ragusa et al., 2021).

Numerous studies have shown that marine animals frequently ingest MPs and that MPs have severe effects on them due to aggregation in the epidermis, acting as bacterial transport channels and absorbing chemical compounds on their surface (Auta et al., 2017; Kumar et al., 2022; Roch et al., 2020; Rubin et al., 2021). In vitro studies have demonstrated that polystyrene microplastics can increase oxidative stress and inflammatory responses in lung epithelial cells, impair pulmonary barrier function, and potentially adversely affect respiratory health (Dong et al., 2020). Polypropylene MPs are present in the human placenta (Ragusa et al., 2021).

Plastics cover corals, sponges, and animals living on the seafloor, depriving them of light, nutrients, and oxygen, depleting the sedimentary environment of oxygen, and reducing the number of organisms (Green et al. 2017; Balestri et al. 2017). This can negatively affect ecosystems and create an environment conducive to pathogens that harm marine life. In addition, endocrine disruptors, one of the most dangerous chemical pollutants in plastics, affect hormones and cause reproduction, development, and behavioral disorders in many marine organisms (Porte et al. 2006). Even some plastics that do not threaten food safety can be highly toxic to aquatic organisms and humans (Hamlin et al. 2015; Muncke et al. 2020). Long-lived substances such as polychlorinated biphenyls (PCBs) adversely affect organisms and environmental health (Geyer et al. 2017). Since they do not degrade, they can be transported over long distances by wind and water and can leave long-lasting effects even far away from their point of origin (Tekman et al. 2022).

Numerous studies have demonstrated the widespread occurrence of MPs in commercially important fish species from marine ecosystems, highlighting their potential ecological and food safety implications. In Türkiye, Gündoğdu and Çevik (2019) reported the presence of MPs in 44.3% of fish collected from the Marmara, Aegean, and Mediterranean Seas, with an average abundance of 1.08 MP particles per fish. In addition, MPs were detected in 18.8% of red shrimp samples and 91.2% of stuffed mussels. Polymer analysis identified 13 different plastic types, with PE, PP, and PET being the most frequently detected polymers, reflecting the widespread use of these materials in packaging and consumer products.

Similarly, Güven et al. (2017) investigated 1,337 individuals representing 28 fish species collected from ten locations in the eastern Mediterranean Sea. They

detected a total of 1,822 MP particles in the digestive tracts of 58% of the examined fish. Red mullet (*Mullus barbatus*) and horse mackerel (*Trachurus trachurus*) exhibited the highest levels of MP contamination among the investigated species, suggesting species-specific differences associated with feeding behavior, habitat preference, and trophic ecology.

Microplastic contamination has also been extensively documented in the Black Sea. According to the TÜBİTAK-MAM and Ministry of Environment and Urbanization (TÜBİTAK MAM and MoEU, 2017) monitoring study, MP concentrations in surface waters reached 3,248,857 particles/km² in 2015 and increased to 8,310,431 particles/km² in 2016. In the water column, MP concentrations were reported as 91.88 particles/m³ in 2015 and 62.44 particles/m³ in 2016, whereas sediment samples contained 3,780 and 11,760 particles/L in 2015 and 2016, respectively. Examination of the gastrointestinal tracts of 263 fish belonging to three commercially important species (*Trachurus trachurus, Merlangius merlangus*, and *Mullus barbatus*) revealed that 174 individuals (66%) had ingested MPs. Seventeen different plastic types, including fibers, hard plastics, and nylon fragments, were identified, demonstrating the widespread distribution of plastic debris throughout different compartments of the Black Sea ecosystem.

Comparable findings have been reported from other Mediterranean regions. Di Giacinto et al. (2023) investigated edible muscle tissues of Mediterranean swordfish (*Xiphias gladius*) and Atlantic bluefin tuna (*Thunnus thynnus*), identifying MPs together with polymer-associated compounds such as PET, PC, bisphenol A (BPA), and p-phthalic acid (PTA). MP abundance ranged from 140 to 270 particles kg^{-1} in swordfish and from 160 to 270 particles kg^{-1} in tuna. Polypropylene was the dominant polymer in both species, accounting for approximately one-third of the detected particles. Although differences in particle morphology, colour, pigments, and polymer composition were observed between the two fish species, the overall levels of plastic contamination were comparable.

Likewise, Digka et al. (2018) investigated MPs contamination in Mediterranean mussels (*Mytilus galloprovincialis*) and three commercially important fish species (*Sardina pilchardus, Pagellus erythrinus,* and *Mullus barbatus*) from the Northern Ionian Sea. MPs were detected in 46.3% of mussels and in all examined fish species, with the highest occurrence observed in *S. pilchardus* (47.2%). The abundance of ingested microplastics ranged from 1.7 to 2.0 particles per individual in mussels and from 1.5 to 1.9 particles per individual in fish. Fragment-shaped particles were the predominant form, while polyethylene was identified as the most common polymer type. These findings provide baseline evidence of Mp contamination in commercially important seafood species from the Ionian Sea.

Similarly, Renzi (2018) reported MP ingestion in small pelagic fish, detecting an average of 4.63 particles per individual in European sardine (*Sardina pilchardus*) and 1.25 particles per individual in European anchovy (*Engraulis encrasicolus*). Sardines exhibited significantly higher MP loads than anchovies,

suggesting that feeding strategy and ecological niche may influence the extent of MP ingestion.

Kılıç (2024) investigated the occurrence of microplastics in the gills and gastrointestinal tracts of eight commercially important Mediterranean fish species, including *Boops boops, Mullus barbatus, Mullus surmuletus, Saurida undosquamis, Sardina pilchardus, Trachurus mediterraneus, Pagellus erythrinus, Oblada melanura*, and *Diplodus annularis*. The mean abundance of MPs was 0.8 ± 1.2 particles per individual in the gastrointestinal tract and 0.3 ± 0.7 particles per individual in the gills. Fibers (79%) were the predominant particle type, followed by black (39%) and blue (37%) particles, while most MPs were smaller than 500 μm (63%). Fourier transform infrared spectroscopy (FTIR) identified polyethylene, polyethylene derivatives, and polypropylene as the dominant polymer types. Ecological risk assessment based on the polymer risk index indicated low to moderate hazard levels, with demersal and benthopelagic fish species exhibiting greater susceptibility to MP-associated toxicity than pelagic species.

More recently, Şimşek (2025) evaluated microplastic contamination in the gastrointestinal tracts of six commercially important fish species collected from Samsun in the Middle Black Sea Region, including *Oncorhynchus mykiss, Dicentrarchus labrax, Sparus aurata*, *Mullus barbatus*, *Trachurus mediterraneus*, and *Merlangius merlangus*. Microplastics were detected in all examined species, with abundances ranging from 4.73 ± 1.13 to 9.26 ± 2.18 particles per individual, the highest levels being observed in *O. mykiss* and the lowest in *M. merlangus*. Particles smaller than 100 μm represented 48.9% of all detected microplastics, while fibers (45.7%) and fragments (36.5%) were the predominant morphological forms. Polymer characterization revealed that polypropylene (31.3%) and polyethylene (23.9%) were the most abundant polymer types. These findings demonstrate the widespread occurrence of microplastics in commercially important fish species from the Middle Black Sea and highlight their potential implications for marine ecosystem health and seafood safety.

The European Food Safety Authority (EFSA, 2016) has also summarized evidence demonstrating the widespread occurrence of MPs in marine fish and shellfish intended for human consumption. Reported contamination levels reached up to seven particles per fish and approximately ten particles per gram of shellfish, with particle sizes ranging from 0.13 to 5 mm.

Overall, available evidence consistently demonstrates that MPs are widely distributed throughout marine ecosystems and are frequently detected in fish species of commercial importance. Polyethylene, polypropylene, and polyethylene terephthalate are the dominant polymers, while fibers and fragments represent the most common particle morphologies. The widespread occurrence of MPs in edible fish tissues and gastrointestinal tracts highlights their potential to transfer plastic-associated contaminants through aquatic food webs and ultimately contribute to human dietary exposure via seafood consumption.

1.5. Human Exposure and Health Risks

The occurrence of MPs in edible fish has raised increasing concerns regarding food safety and public health. In addition to the particles themselves, the associated chemical additives and adsorbed environmental contaminants may be transferred through aquatic food webs and ultimately reach humans via seafood consumption. Although important uncertainties remain regarding the long-term health consequences of dietary exposure, current evidence highlights the need for continued environmental monitoring, standardized analytical methods, and comprehensive risk assessments to better understand the ecological and human health implications of microplastic pollution.

Hydrophobicity, chemical structure (surface charge), and particle size of microplastics adversely affect human health. MPs cannot pass through cell membranes due to their size. However, they cause inflammation in the intestine and thus negatively affect the immune system. In a study on rodents, it was found that MPs were very difficult to be absorbed in the blood and a very small amount of microplastics could reach the bloodstream through the lymph, but this amount could not penetrate the organs and was probably eliminated through the spleen (Bouwmeester et al., 2015). In another study, it was found that most of the ingested MPs and nanoplastics were excreted from the human body (Smith et al., 2018). The potential effects of MPs on human health depend more on particle properties, and it has been observed that nanoplastics, rather than microplastic particles, adversely affect human health. MPs have been detected in stool samples from people living in different countries (Rist et al., 2018). Nanoplastics can penetrate all organs, including the placenta and brain, and can be transported into cell membranes (Bouwmeester et al., 2015).

The adverse effects of MPs on human health vary according to their chemical structure. For example, PET is considered potentially carcinogenic to humans. They are widely used in the production of beverage bottles, plastic film, microwave packaging, pipes, and insulation moulds. Additives used to make PVC can be transferred from medical equipment to humans and accumulate in the blood (Karbalaei et al. 2018). Bisphenol A (BPA) is a monomeric compound of PC and is among the endocrine-disrupting chemicals and has toxic effects on brain and nerve cells. Humans are exposed to it through inhalation, dermal contact, and ingestion, and as a result it can be found in urine, blood, breast milk, and tissue samples. Prenatal exposure to bisphenol A has been associated with behavioural and emotional disturbances in offspring, while accumulating evidence also suggests adverse effects on female and male reproductive health (Rist et al., 2018; Siracusa et al., 2018). Phthalates are also found in plastic products [such as din-octyl phthalate (DnOP) and di(2-ethylhexyl) phthalate (DEHP)] and are used as plasticizers to increase flexibility and durability. Butyl benzyl phthalate (BBP) and di-2-ethylhexyl phthalate (DEHP) are classified as probable human carcinogens (Karbalaei et al., 2018). Phthalates are thought to cause developmental abnormalities and have been found to have effects on male reproductive health, pregnancy, and respiratory health (Rist et al., 2018). Polybrominated diphenyl ethers (PBDE) and tetrabromobisphenol A (TBBPA),

which are additives used as flame retardants in plastic products, disrupt the activity of thyroid hormone, and PBDEs cause hormone disruption in humans (Rist et al., 2018).

Since microplastics are highly absorbent, they attract pollutants such as heavy metals, endocrine-disrupting chemicals, and persistent organic pollutants (Yurtsever, 2015; GESAMP, 2015; Guo and Wang, 2019). These harmful substances cause harmful effects such as birth defects, cognitive development disorders, reproductive problems, and cancer (Smith et al., 2018). It has also been discovered that microorganisms, including pathogens, are associated with MPs (Syranidou et al. 2017; Brandon et al. 2018). Bacterial groups detected in MPs include *Bacillus* spp., *Paenibacillus* spp., *Actinobacteria* and *Firmicutes* (Lwanga et al. 2018; Park and Kim 2019). Although MP can persist in the environment and resist degradation, some microplastic-associated bacteria can degrade MP (Alimi et al. 2018; Kim et al. 2017; Sen and Raut 2015; Yuan et al. 2020). Different bacterial consortia exhibit varying microplastic degradation capabilities depending on bacterial species and enzymes (Tsiota et al. 2018).

Conclusion

Microplastic pollution has emerged as one of the most significant environmental challenges affecting aquatic ecosystems worldwide. The studies reviewed demonstrate that microplastics are widely distributed in marine and freshwater environments and have been detected in numerous commercially important fish species regardless of habitat, feeding behavior, or geographic region. Fibers and fragments are consistently reported as the dominant particle morphologies, while polyethylene, polypropylene, and polyethylene terephthalate are the most frequently identified polymer types.

Current evidence indicates that microplastic ingestion may adversely affect fish through oxidative stress, inflammation, metabolic disturbances, impaired growth, reproductive dysfunction, behavioral alterations, and immune suppression. In addition, microplastics may act as vectors for hazardous chemicals, heavy metals, and pathogenic microorganisms, thereby amplifying their ecological and toxicological impacts. The widespread occurrence of microplastics in edible fish species also raises concerns regarding human dietary exposure. Although most ingested microplastics appear to be excreted from the human body, smaller particles, particularly nanoplastics, may cross biological barriers and interact with tissues, highlighting the need for further investigation into the long-term health consequences of chronic exposure.

Despite the rapid increase in research on microplastics, considerable methodological differences among studies—including sampling procedures, extraction methods, polymer identification techniques, and data reporting—limit direct comparisons and comprehensive risk assessments. Therefore, harmonized analytical protocols, standardized reporting criteria, and internationally coordinated monitoring programs are essential to improve the reliability and comparability of future studies.

Future research should prioritize the occurrence and biological effects of smaller microplastics and nanoplastics, their trophic transfer within aquatic food webs, interactions with co-occurring environmental contaminants, and their accumulation in edible fish tissues. Integrating environmental monitoring with food safety, toxicological, and epidemiological studies will be crucial for accurately assessing human exposure and health risks. Adopting a One Health approach that recognizes the interconnectedness of environmental, animal, and human health will be fundamental for developing effective strategies to mitigate microplastic pollution, safeguard aquatic ecosystems, and protect public health.

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CHAPTER 12

Food Noise in Eating Behaviour: Conceptual Boundaries, Measurement, and Implications for Nutrition and Dietetics

***Sedef Güngör*[1]**

[1] Dr. Öğr. Gör., Atılım Üniversitesi, ORCID: 0000-0002-2338-8576

1. Introduction

Food-related thoughts are a normal part of everyday life; however, in some individuals, food-related cognition may become repetitive, mentally dominant, difficult to disengage from, or burdensome. The term "food noise" is increasingly used to describe persistent, intrusive, unwanted, or disruptive thoughts about food (Dhurandhar et al., 2025; Diktas et al., 2025). Public and clinical use of the term preceded the development of an agreed scientific definition, and its visibility increased as some individuals using glucagon-like peptide-1 receptor agonists (GLP-1 RAs) reported marked reductions in persistent food-related thoughts. Nevertheless, scientific approaches have not defined food noise solely as an outcome of pharmacological treatment (Dhurandhar et al., 2025; Hayashi et al., 2023).

Although the term captures a subjective experience that some individuals can readily identify, it enters a broader literature in which related constructs such as food craving, food preoccupation, hedonic hunger, food cue reactivity, intrusive cognition, rumination, and addiction-like eating are already well established. Its scientific value therefore depends on demonstrating the extent to which food noise can be conceptually and psychometrically distinguished from these neighbouring constructs and whether it provides incremental information beyond them (Brewis et al., 2026; Dhurandhar et al., 2025).

From a nutrition and dietetics perspective, food noise may be relevant because it may intersect with processes routinely addressed in dietetic practice, including appetite, food craving, dietary restraint, meal planning, and treatment adherence. However, the extent to which food noise can be distinguished from neighbouring eating-behaviour constructs remains unclear, and the concept should not be interpreted in ways that medicalise ordinary food-related cognition (Brewis et al., 2026). Accordingly, this chapter approaches food noise not as an established clinical disorder but as an emerging construct, examining its conceptual boundaries, measurement, possible mechanisms, and implications for dietetic practice. Findings relating to GLP-1 RAs are considered not to evaluate treatment efficacy, but insofar as they contribute to the conceptualisation of food noise and to understanding its possible mechanisms.

2. From Popular Expression to Scientific Construct

Food noise entered scientific discussion after first appearing in patient and public discourse. Dhurandhar et al. (2025) were unable to determine the exact origin of the term; however, following earlier internet search activity, they reported a marked increase in interest from late 2022 to 2024, coinciding with reports from GLP-1 RA users of reductions in persistent food-related thoughts. Current descriptions focus on repetitive mental activity related to food, eating decisions, calories, meal timing, and anticipated eating opportunities. Thus, although the experience itself may be older, the label may be relatively new; some

individuals may only have recognised the experience after it diminished during treatment.

Early scientific approaches addressed this experience from different theoretical starting points. Hayashi et al. (2023) conceptualised food noise within the Cue–Influencer–Reactivity–Outcome (CIRO) framework as intensified or prolonged food-cue reactivity that may lead to intrusive food-related thoughts and maladaptive eating behaviours. Diktas et al. (2025), who developed the Food Noise Questionnaire (FNQ), instead emphasised persistent and intrusive food-related cognition that may occur even in the absence of identifiable external cues, while noting that cues may nevertheless trigger or intensify the experience.

In the same year, Dhurandhar et al. (2025) proposed a formal definition: "persistent thoughts about food that are perceived by the individual as being unwanted and/or dysphoric and may cause harm to the individual, including social, mental, or physical problems". This framework emphasised cognitive burden, persistence, dysphoria, and self-stigma, and later provided the conceptual basis for the Ro Allison Indiana Dhurandhar Food Noise Inventory (RAID-FN) (Dhurandhar et al., 2026).

Thus, within only a few years, different conceptualisations of food noise have emerged. Nevertheless, the terminology and conceptual boundaries remain incompletely established (Brewis et al., 2026). The following section therefore positions food noise within established eating-behaviour constructs before considering possible mechanisms and measurement.

3. Conceptual Boundaries

The scientific value of food noise depends, in part, on the extent to which it can be clearly positioned within the established literature on food-related thoughts, motivation, and behavioural responses. Although current approaches converge in emphasising repetitive food-related cognition, they differ in the weight they place on food cues, persistence, unwantedness, distress, cognitive burden, interference with daily functioning, and self-stigmatising experiences (Dhurandhar et al., 2025; Diktas et al., 2025; Hayashi et al., 2023). These differences are important because the extent to which food noise can be distinguished from neighbouring constructs has not yet been fully established. Brewis et al. (2026) similarly identified theoretical grounding, conceptual overlap, and construct distinctiveness as key unresolved issues.

3.1. Food Craving

Food craving has a longer empirical history and is generally conceptualised as an intense desire to consume a specific food or type of food (Meule, 2020). It may occur as a transient state experienced at a particular moment or as a more enduring, person-specific tendency (Cepeda-Benito, Gleaves, Williams, & Erath, 2000).

Food craving and food noise may overlap in repetitive food-related thoughts and difficulty disengaging from them. However, whereas food craving centres on the desire to consume, food-noise approaches describe persistent cognition that need not be directed towards a specific food and characterise it in terms of unwantedness, cognitive burden, and interference with daily life (Dhurandhar et al., 2025; Diktas et al., 2025). The extent to which this distinction is empirically valid has not yet been established.

3.2. Food Preoccupation

Food preoccupation is one of the closest conceptual neighbours of food noise. Tapper and Pothos (2010) considered food preoccupation not only in terms of the frequency of food-related thoughts but also in terms of their affective valence, emphasising that frequent thoughts about food may be positive, negative, or neutral.

Frequent thinking about food is not inherently negative; it may relate to anticipation, planning, professional activity, or pleasure. Food-noise approaches, by contrast, place greater emphasis on persistence, difficulty disengaging from thoughts, interference with daily life, and dysphoria (Dhurandhar et al., 2025; Diktas et al., 2025). Food noise may therefore, in some circumstances, be understood as a form of food preoccupation in which thoughts are experienced as unwanted, persistent, or disruptive; however, this distinction has not yet been demonstrated empirically.

3.3. Hedonic Hunger

Hedonic hunger refers to appetite and motivation driven by the anticipated pleasure of palatable food rather than by immediate physiological need (Lowe & Butryn, 2007). The concept also encompasses the psychological effects of environments in which palatable foods are continuously available even when food is not being consumed (Lowe et al., 2009). Espel-Huynh, Muratore, and Lowe (2018) described hedonic hunger as involving thinking about and wanting palatable food for its pleasurable properties when the body is not physiologically hungry.

Food noise may overlap with hedonic hunger in food-related thoughts that occur in the absence of physiological hunger. However, whereas hedonic hunger is organised around reward and anticipated pleasure from eating, food-noise approaches emphasise persistence, intrusive cognition, cognitive burden, and dysphoria (Dhurandhar et al., 2025; Diktas et al., 2025). The extent of overlap between the two constructs remains unclear.

3.4. Food Cue Reactivity

Food cue reactivity refers to physiological, cognitive, and behavioural responses triggered by food-related cues such as the sight or smell of food

(Boswell & Kober, 2016). Food noise is closely related to this construct; Hayashi et al. (2023) conceptualised food noise directly within a food-cue-reactivity framework.

However, not all definitions of food noise require cue exposure. Diktas et al. (2025) noted that food noise may occur in the absence of identifiable external cues, although cues may initiate or intensify the experience. The conceptual boundary between food cue reactivity and food noise therefore remains incompletely established.

3.5. Intrusive Food-Related Cognitions

Intrusive food-related cognition represents another close conceptual neighbour of food noise. Elaborated Intrusion theory distinguishes spontaneously arising thoughts from their subsequent elaboration through attention and imagery (Kavanagh, Andrade, & May, 2005; May, Kavanagh, & Andrade, 2015).

Food noise may similarly involve intrusive thoughts, difficulty disengaging from them, and cognitive burden. However, this overlap does not establish that food noise can be reduced to elaborated food craving. Dhurandhar et al. (2025) likened more intense food noise to rumination; rumination, however, represents a broader form of repetitive thinking and is not food-specific (Nolen-Hoeksema, Wisco, & Lyubomirsky, 2008). How food noise differs from rumination and obsessive thinking remains unclear (Dhurandhar et al., 2026).

3.6. Food Addiction

Food addiction is a broader construct than repetitive food-related cognition. It refers to an addiction-like pattern of eating defined using criteria adapted from substance-related and addictive disorders. These criteria include impaired control over eating, continued consumption despite adverse consequences, craving, and clinically significant impairment or distress (Gearhardt, Corbin, & Brownell, 2016). Gearhardt and Schulte (2021) reviewed biological and behavioural evidence relevant to applying an addiction framework to highly processed foods while emphasising that conceptual and methodological debates remain ongoing.

Food noise may overlap with addiction-like eating through craving, preoccupation, perceived loss of control, and cognitive burden. However, whereas food addiction describes a broader behavioural and clinical pattern, food noise refers to a cognitive experience that may occur in the absence of that broader pattern (Dhurandhar et al., 2026).

Overall, food noise occupies a dense conceptual space that includes food craving, food preoccupation, hedonic hunger, food cue reactivity, intrusive cognition, rumination, and addiction-like eating. Persistence, unwantedness, dysphoria, cognitive burden, and interference with daily life may represent distinguishing features; however, these boundaries have not yet been

demonstrated consistently (Brewis et al., 2026; Dhurandhar et al., 2026; Diktas et al., 2025).

4. Competing Explanatory Models and Possible Mechanisms

Recognition of food noise as a subjective experience does not imply that it has a unique underlying mechanism. Current evidence is compatible with several possible pathways, including food cue reactivity, intrusive cognition, dietary restriction, and homeostatic or reward-related signalling; however, none of these processes has been shown to explain food noise specifically (Brewis et al., 2026; Dhurandhar et al., 2025).

4.1. Cue Reactivity and Conditioned Responding

Cue-based explanations draw on associative learning. Repeated pairings among environments, routines, emotions, and eating may allow cues to trigger anticipatory responses even in the absence of immediate energy need. Food cue reactivity may involve both cognitive and physiological responses, and these responses have been shown to be related in adults with overweight or obesity (Kang Sim, Eichen, Strong, Manzano, & Boutelle, 2023).

Such conditioned responses provide one possible explanation for why food-related thoughts may become more frequent and difficult to disengage from. Food cue reactivity should nevertheless be regarded as one possible contributor rather than as a complete explanation of food noise.

4.2. Intrusive Cognition, Attentional Capture, and Elaboration

Another possible pathway concerns how spontaneously arising food-related thoughts enter awareness and remain active. According to Elaborated Intrusion theory, intrusive thoughts may arise from physiological states, external cues, affect, or associative processes; when they signal anticipated pleasure or relief, attention and sensory imagery may elaborate and sustain desire (Kavanagh et al., 2005; May et al., 2015).

Because elaboration competes for working-memory resources, this model may help explain why transient food-related thoughts can become salient and mentally demanding. Desire-related imagery may also become distressing when consumption is not possible or when the desired outcome conflicts with another goal, providing a possible pathway towards dysphoric experience.

This is not, however, a demonstrated mechanism of food noise, but rather a possible explanatory analogy. Studies examining intrusive-thought frequency, imagery, attentional bias, and repetitive thinking alongside food-noise measures are needed (Dhurandhar et al., 2026).

4.3. Dietary Restriction, Deprivation, and Cognitive Restraint

Dietary restriction represents another plausible but complex pathway. The food-craving literature does not support a simple linear relationship: short-term avoidance of a specific food may increase craving for that food, whereas longer-term energy restriction in adults with overweight has more often been followed by reductions in craving (Meule, 2020). Physiological deprivation, selective avoidance, and cognitive restraint should therefore not be treated as equivalent exposures.

Food-noise research points to a similar distinction. Individuals who were currently dieting reported greater food noise than those who were not dieting (Diktas et al., 2025), whereas trait-level dietary restraint showed only a weak association with food noise (Dhurandhar et al., 2026). Rather than being contradictory, these findings may reflect different dimensions of restriction.

Dhurandhar et al. (2025) also reported an anecdotal observation that food-related cognitive burden increased during the first days of a low-energy diet and subsequently declined. This is not causal evidence but a hypothesis-generating observation; nevertheless, it is consistent with evidence that short- and longer-term restriction may relate differently to food craving (Meule, 2020).

4.4. Homeostatic, Hedonic, and Reward-Related Signalling

Food-related cognition is embedded within physiological systems that regulate appetite, satiation, satiety, reward, and motivation. Because endogenous GLP-1 and pharmacological GLP-1 receptor agonists influence several of these processes, GLP-1 signalling is relevant in this context (Dhurandhar et al., 2025; Rutigliani et al., 2026).

In a 20-week randomised trial in adults with obesity, semaglutide 2.4 mg reduced energy intake at an ad libitum lunch by approximately 35% compared with placebo and was associated with lower hunger and prospective food consumption, greater perceived control of eating, and less frequent and less intense food cravings (Friedrichsen, Breitschaft, Tadayon, Wizert, & Skovgaard, 2021).

Rutigliani et al. (2026) reviewed eight candidate mechanisms that may contribute to GLP-1 RA-associated weight loss and did not identify a single dominant mechanism. Food noise was considered separately from appetite and craving. The review specifically considered appetite, chemosensory function, food cravings and aversions, food noise, gastric emptying, the gut microbiome, incretin activity, and energy expenditure.

Human studies also suggest that GLP-1 RAs may reduce energy intake and cortical responses to rewarding stimuli, particularly energy-dense food cues (Badulescu et al., 2024). These findings are compatible with links among appetite

signalling, reward, and food-related cognition, but they do not demonstrate a mechanism specific to food noise.

4.5. What GLP-1 Receptor Agonists Can—and Cannot—Tell Us About Food Noise

Reports of reductions in food noise during GLP-1 RA treatment have contributed to the contemporary visibility of the concept; however, a pharmacological response does not establish either construct distinctiveness or mechanism (Brewis et al., 2026; Dhurandhar et al., 2025).

The trials summarised by Dhurandhar et al. (2025) assessed hunger, satiety, craving, and control of eating rather than food noise itself. GLP-1 RA findings should therefore be interpreted not as a pharmacological definition of food noise, but as evidence that may generate hypotheses regarding possible physiological mechanisms.

Studies that directly measure food noise alongside hunger, craving, body weight, and related eating constructs are needed to determine whether treatment-related changes are specific to food noise or form part of broader appetite regulation.

4.6. Toward an Integrative Model

A provisional, integrative model appears more plausible than a single-mechanism explanation. Persistent food-related cognition may arise from interactions among appetite signals, learned cue associations, reward valuation, spontaneously arising cognitive intrusions, and efforts to regulate eating, with the relative contribution of these components varying across individuals and situations.

Internal states or external cues may activate learned food representations that enter awareness and acquire motivational or emotional salience. Additional attention, imagery, monitoring, suppression, or conflict with dietary goals may increase persistence and cognitive burden. Such an account allows cues to exert an influence without being necessary and recognises that restriction may either increase or reduce food salience depending on context.

The model remains hypothetical. Mechanistic studies should move beyond cross-sectional self-report associations and test whether food noise provides incremental predictive or explanatory information after established mechanisms and neighbouring constructs are taken into account (Brewis et al., 2026).

5. Measurement of Food Noise

Until recently, food noise was described largely through patient narratives, media discourse, and clinical observations. Dhurandhar et al. (2025) noted that the absence of both a formal definition and a validated measurement instrument

limited efforts to characterise the construct and track changes over time. Two independent measures were subsequently developed: the Food Noise Questionnaire (FNQ; Diktas et al., 2025) and the Ro Allison Indiana Dhurandhar Food Noise Inventory (RAID-FN; Dhurandhar et al., 2026). Because these instruments operationalise food noise from different conceptual frameworks, they should not be treated as interchangeable versions of the same measure.

5.1. The Food Noise Questionnaire

The FNQ is a five-item measure assessing persistent and disruptive food-related thoughts. Items are rated on a 0–4 scale with reference to the previous two weeks. In its US validation study, the instrument showed a unidimensional structure, high internal consistency, and adequate test–retest reliability (Diktas et al., 2025).

FNQ scores were strongly correlated with the frequency subscale of the Food Preoccupation Questionnaire (FPQ; Tapper & Pothos, 2010) and the preoccupation-related subscale of the Food Cravings Questionnaire–Trait (Cepeda-Benito et al., 2000). These findings support convergent validity, but they also raise the question of how clearly food noise can be distinguished from existing constructs. In addition, the validation study did not directly test whether unwantedness or negative affective valence distinguishes food noise from ordinary food preoccupation (Diktas et al., 2025).

5.2. The RAID-FN

The RAID-FN was developed from a conceptualisation of food noise as persistent food-related thoughts perceived as unwanted and/or dysphoric (Dhurandhar et al., 2025). The validation process resulted in a 23-item long form and a seven-item short form, with a three-factor structure comprising food preoccupation, persistence, and dysphoria. The short form showed high internal consistency and high test–retest reliability at the total-score level, although reliability was lower for the dysphoria subscale (Dhurandhar et al., 2026).

RAID-FN scores showed strong associations with general food craving and food cue responsivity. The validation study also identified susceptibility to response bias, leading the authors to emphasise that scores should not be interpreted in isolation, particularly in clinical decision-making contexts (Dhurandhar et al., 2026).

Dhurandhar et al. (2026) compared RAID-FN and FNQ in terms of model fit and convergent validity. However, direct comparison is limited because the two studies used different model characteristics, criterion measures, and correlation methods. In particular, the relatively high root mean square error of approximation (RMSEA) reported for the FNQ should be interpreted in light of the known limitations of this index in models with very low degrees of freedom (Kenny, Kaniskan, & McCoach, 2015; Mocini et al., 2026).

Overall, both instruments demonstrate good reliability, but both also overlap substantially with neighbouring constructs such as food craving and food preoccupation, and neither has a validated diagnostic cut-off (Dhurandhar et al., 2026; Diktas et al., 2025). Current evidence is therefore stronger for reliability and convergent validity than for discriminant and incremental validity. Whether food-noise scores provide additional clinical or explanatory information beyond established constructs remains uncertain.

6. Implications for Nutrition and Dietetics Practice

Food noise may be relevant to dietetic practice because persistent food-related cognition can intersect with appetite, food cravings, dietary restriction, meal planning, and treatment adherence. However, direct evidence for dietetic interventions specifically targeting food noise remains limited. Dhurandhar et al. (2025) framed questions concerning dietary adherence and lifestyle strategies primarily as research priorities rather than established clinical guidance. Accordingly, the implications outlined below are intended to extend existing principles of assessment and counselling rather than to propose a separate treatment protocol.

6.1. Incorporating Food Noise into Dietetic Assessment

Food noise may warrant consideration when food-related thoughts are persistent, distressing, difficult to disengage from, or disruptive to daily functioning. Assessment should focus less on assigning a label and more on the characteristics and context of the experience. Frequency, unwantedness, associations with hunger or craving, interference with daily life, emotional burden, and links with dietary restriction should be considered together (Dhurandhar et al., 2026; Diktas et al., 2025).

Frequent thoughts about food alone are unlikely to indicate clinical significance, as they may occur in contexts such as restrictive dieting, intensive meal planning, eating-disorder symptoms, or treatments that alter appetite. Existing measures may therefore support structured assessment but should not be used diagnostically; rather, they should complement dietary history, experiences of hunger and satiety, eating behaviour, psychological context, and clinical judgement (Dhurandhar et al., 2026; Diktas et al., 2025).

6.2. Dietary Restriction and the Structure of Nutrition Counselling

Different forms of dietary restriction may affect food-related cognition in different ways (Meule, 2020). It should therefore not be assumed that either greater dietary control or less restriction will reduce food noise in all individuals.

Rigid dietary rules, selective avoidance, or perceived deprivation may sustain attention to food in some individuals (Diktas et al., 2025; Polivy & Herman, 2017; Tapper & Pothos, 2010), whereas a structured and sustainable eating pattern may

be followed by fewer cravings in others (Meule, 2020). A key dietetic question is whether the current dietary approach is creating unnecessary cognitive burden and whether the same nutritional goals could be achieved through a more sustainable structure.

6.3. Counselling Language, Self-Blame, and Stigma

How food noise is labelled and explained may influence self-blame and perceptions of control. Current conceptualisations include unwantedness, dysphoria, and self-stigmatising experiences (Dhurandhar et al., 2025).

Repetitive food-related thoughts should not be framed as evidence of weak willpower, poor motivation, or moral failure. At the same time, explanatory labels may themselves influence blame, stigma, and perceived control in complex ways (Cassin et al., 2019).

Assessment should therefore clarify what an individual means when using the term "food noise". Hunger, craving, repetitive planning, guilt, fear of loss of control, and intrusive thoughts are not equivalent experiences. Frequent food-related thoughts should likewise not be pathologised solely because they are present; their burden, associated distress, functional impact, and behavioural consequences are more informative (Brewis et al., 2026).

6.4. When Broader Assessment or Referral May Be Needed

Persistent food-related cognition may occur alongside eating-disorder symptoms or other psychological difficulties. Dhurandhar et al. (2025) noted similarities between some experiences described as food noise and those reported in eating disorders. Food noise itself, however, is not an eating-disorder diagnosis, and current food-noise measures should not replace established assessment tools.

More comprehensive assessment or referral may be appropriate when repetitive food-related thoughts occur alongside binge eating, compensatory behaviours, severe restriction, marked fear of weight gain, body-image disturbance, nutritional inadequacy, or clinically significant distress or functional impairment. Similar consideration may be warranted when food-related cognition is closely linked with anxiety, depressive symptoms, or obsessive thinking. Reported associations with these variables do not establish causality or indicate a distinct psychiatric disorder (Dhurandhar et al., 2026; Diktas et al., 2025).

6.5. Potential Strategies: What Can and Cannot Currently Be Recommended

To date, no behavioural or dietary intervention has been validated specifically for food noise. Some established approaches may be theoretically relevant, but evidence from related constructs should not be transferred directly to food noise without empirical testing.

For example, research based on Elaborated Intrusion theory suggests that some strategies targeting attention and imagery may influence intrusive thoughts and craving-related processes (May et al., 2015). Mindful eating and intuitive eating have also been evaluated within the broader eating-behaviour literature; however, a systematic review of randomised trials found limited and inconsistent evidence for effects on energy intake and diet quality (Grider, Douglas, & Raynor, 2021). Whether these approaches specifically reduce food noise remains unknown.

Reductions in food noise may also be reported during GLP-1 RA treatment (Dhurandhar et al., 2025); however, such observations do not establish food noise as a distinct disorder, and the role of food-noise measures in treatment selection or monitoring has not yet been determined.

Given the current evidence, food noise is best treated as a descriptive feature of cognitive experience. Dietetic care should therefore avoid assumptions about food-noise-specific treatment effects and instead address identifiable nutrition- and eating-related difficulties using established approaches.

7. Future Directions

Food-noise research has progressed rapidly from anecdotal descriptions to formal definitions and dedicated measurement instruments. However, the extent to which the construct can be distinguished from established eating-behaviour concepts, its variability over time, and its clinical relevance remain insufficiently understood (Brewis et al., 2026; Dhurandhar et al., 2025). Future studies should therefore examine whether food noise provides additional explanatory or predictive value beyond established constructs such as food craving, food preoccupation, hedonic hunger, food cue reactivity, and dietary restraint.

Research should also move beyond cross-sectional self-report designs. Ecological momentary assessment, repeated-measures approaches, and longitudinal studies could clarify how food noise varies in relation to hunger, stress, food cues, eating episodes, and dietary restriction. Likewise, pharmacological, dietary, behavioural, and cognitive intervention studies should measure food noise directly to determine whether observed changes are distinct from changes in neighbouring processes such as appetite or craving.

From a nutrition and dietetics perspective, the key question is whether food-noise measures provide meaningful information beyond existing assessments in relation to dietary adherence, perceived dietary difficulty, quality of life, or clinician–patient communication. Ultimately, the clinical value of the construct will depend not simply on its correlations with other variables, but on whether it adds useful information to assessment and care.

8. Conclusion

Food noise is an emerging concept that has rapidly moved from patient and public discourse into scientific research. Current evidence suggests that it may be useful for describing persistent and burdensome food-related cognition, but the extent to which it can be distinguished from neighbouring constructs such as food craving, food preoccupation, hedonic hunger, and intrusive cognition remains unclear. Possible mechanisms include food cue reactivity, appetite and reward signalling, dietary restriction, and cognitive processes, although none has been shown to be specific to food noise. From a nutrition and dietetics perspective, the concept may currently be most useful as a descriptive framework for identifying cognitive burden that may be overlooked when assessment focuses primarily on food intake. A key priority for future research is to determine whether food noise provides additional explanatory and clinical value beyond established constructs.

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CHAPTER 13

Approach to Inflammatory Joint Pain

***Ali Ekin*[1]**

[1] Assoc. Prof., Van Yüzüncü Yıl University, Faculty of Medicine, Department of Internal Medicine, Division of Rheumatology, ORCiD: 0000-0003-3692-1293

1. Introduction

Joint pain is among the most frequent musculoskeletal complaints encountered in clinical practice. The fundamental initial task is to determine whether the symptoms arise from an inflammatory articular process, a non-inflammatory structural disorder, periarticular disease, or a non-musculoskeletal source. This distinction is clinically important because inflammatory arthritis encompasses disorders with substantially different prognoses and therapeutic implications.

Inflammatory arthritis is not itself a diagnosis. It represents a clinical syndrome characterized by inflammation involving synovial joints and, depending on the underlying disease, adjacent structures such as tendon sheaths, entheses, bursae, or the axial skeleton. Rheumatoid arthritis (RA), psoriatic arthritis (PsA), axial and peripheral spondyloarthritis (SpA), crystal-induced arthritis, connective tissue diseases, reactive and infection-associated arthritis, and septic arthritis are major diagnostic considerations.

Early recognition is particularly important in persistent inflammatory arthritis. The concept of a therapeutic "window of opportunity" has transformed the approach to early arthritis. Patients with objective joint swelling associated with pain or stiffness should be evaluated promptly, and EULAR recommendations emphasize early rheumatology referral and early disease-modifying treatment in patients at risk of persistent disease [1]. Contemporary management of RA continues to emphasize initiation of disease-modifying antirheumatic drugs (DMARDs) as soon as the diagnosis is established and a treat-to-target strategy thereafter [2].

The diagnostic process should therefore answer three sequential questions:

1. Is the pain truly articular and inflammatory?
2. What is the anatomical and temporal pattern of inflammation?
3. Which disease best explains this pattern and its associated systemic manifestations?

2. Is the Joint Pain Inflammatory?

The traditional distinction between inflammatory and mechanical pain remains useful, although no individual symptom is sufficiently accurate to establish or exclude inflammatory arthritis.

Inflammatory joint pain typically improves with activity, is aggravated by prolonged inactivity, may occur during the night or early morning, and is frequently associated with prolonged morning stiffness. Mechanical pain generally increases with loading or repetitive activity and improves with rest. However, these characteristics overlap substantially. Osteoarthritis can produce

morning stiffness and episodic synovitis, whereas early inflammatory arthritis may initially cause only mild stiffness or intermittent symptoms.

Objective evidence of inflammation is therefore more important than symptom terminology alone.

Feature	Inflammatory pattern	Mechanical/non-inflammatory pattern
Morning stiffness	Usually prolonged, often >30–60 min	Usually brief
Effect of activity	Often improves symptoms	Frequently worsens symptoms
Effect of rest	Stiffness/gelling increases	Usually improves pain
Night pain	Common, particularly late night/early morning	Variable
Joint swelling	Soft, synovial swelling	Bony enlargement more typical
Warmth	May be present	Usually absent
Systemic symptoms	May occur	Usually absent
CRP/ESR	May be elevated	Usually normal
Power Doppler US	May demonstrate active synovitis	Usually absent or limited

Table 1. Clinical features suggesting inflammatory versus predominantly mechanical joint pain

Importantly, normal C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) do **not** exclude inflammatory rheumatic disease. Conversely, elevated acute-phase reactants are not specific for rheumatic inflammation and may reflect infection, malignancy, obesity, tissue injury, or other systemic processes.

3. Confirming That Symptoms Originate From the Joint

Before defining the inflammatory pattern, the clinician should determine whether symptoms originate from the joint itself or from periarticular structures.

True arthritis is suggested by synovial swelling, warmth, effusion, and restriction of both active and passive range of motion. Pain confined primarily to active movement, with relatively preserved passive movement, should raise suspicion for tendon, muscle, bursa, or other periarticular pathology.

Palpation remains essential. Synovitis typically produces a soft or "boggy" swelling rather than the firm osseous enlargement characteristic of osteoarthritis. Particular attention should be directed toward the metacarpophalangeal (MCP), proximal interphalangeal (PIP), wrist, metatarsophalangeal (MTP), knee, and ankle joints.

Musculoskeletal ultrasonography (US) has substantially increased the ability to identify subtle synovitis, tenosynovitis, and enthesitis. US and magnetic resonance imaging (MRI) can detect inflammatory abnormalities not apparent on routine clinical examination, although increased sensitivity comes at the cost of identifying abnormalities that are not necessarily disease-specific [3].

4. Pattern Recognition: The Core of Clinical Diagnosis

Once inflammatory arthritis has been established or strongly suspected, the diagnostic differential can be narrowed considerably by describing the arthritis along several dimensions.

4.1 Number of involved joints

Inflammatory arthritis may be classified clinically as:

- Monoarthritis: one joint
- Oligoarthritis: two to four joints
- Polyarthritis: five or more joints

Acute monoarthritis should immediately raise consideration of septic arthritis and crystal-induced arthritis. Trauma, hemarthrosis, and less frequently initial presentations of inflammatory rheumatic diseases should also be considered.

An asymmetric lower-extremity oligoarthritis suggests SpA, reactive arthritis, PsA, or crystal disease. Symmetric small-joint polyarthritis strongly suggests RA but may also occur in SLE, viral arthritis, PsA, and other systemic disorders.

4.2 Distribution

The anatomical distribution provides additional diagnostic information. RA preferentially involves the MCP, PIP, wrist, and MTP joints. Distal interphalangeal (DIP) involvement is unusual as a dominant manifestation of RA and should prompt consideration of PsA or osteoarthritis. PsA is remarkably heterogeneous and may manifest as asymmetric oligoarthritis, symmetric polyarthritis resembling RA, DIP-predominant disease, arthritis mutilans, enthesitis, dactylitis, or axial disease. The CASPAR criteria incorporate psoriasis, psoriatic nail disease, negative rheumatoid factor, dactylitis, and radiographic new bone formation and remain the established classification framework [4].

Predominant lower-limb asymmetric arthritis, enthesitis, dactylitis, inflammatory back pain, uveitis, psoriasis, or inflammatory bowel disease increases the probability of SpA.

4.3 Symmetry

Symmetry supports but does not establish RA. Early RA can initially be asymmetric, while PsA may evolve into a symmetric polyarthritis.

Consequently, symmetry should be interpreted together with joint distribution, chronicity, serology, and extra-articular findings.

4.4 Temporal pattern

Duration and evolution are equally important.

Acute arthritis, developing over hours to days, is characteristic of crystal arthritis, septic arthritis, and some reactive or viral arthritides.

Subacute arthritis, evolving over several weeks, may occur in early RA, SpA, connective tissue diseases, sarcoidosis, or infection-related arthritis.

Chronic inflammatory arthritis, persisting for more than approximately six weeks, increases the likelihood of RA, PsA, SpA, or another chronic inflammatory rheumatic disease.

The six-week threshold is useful for classification but should never be used to delay evaluation or treatment.

5. History: Looking Beyond the Joints

A high-quality rheumatologic history should actively search for manifestations that connect arthritis with a broader systemic disease.

Skin and nails

Current or previous psoriasis, including scalp, umbilical, retroauricular, and intergluteal lesions, should be sought. Nail pitting, onycholysis, hyperkeratosis, and oil-drop discoloration support PsA.

A history of photosensitive rash, malar eruption, subacute cutaneous lupus, purpura, erythema nodosum, or livedoid lesions may redirect the differential toward systemic autoimmune disease, vasculitis, sarcoidosis, or thrombo-inflammatory disorders.

Eyes

Acute painful red-eye episodes with photophobia suggest anterior uveitis and strongly support SpA in the appropriate musculoskeletal context. Dry eye symptoms may suggest Sjögren syndrome, although sicca symptoms are common and nonspecific.

Gastrointestinal and genitourinary systems

Chronic diarrhea, bloody stool, abdominal pain, weight loss, or known inflammatory bowel disease should raise suspicion for enteropathic arthritis. Recent gastrointestinal or genitourinary infection may precede reactive arthritis.

Mucosal manifestations

Recurrent oral or genital ulceration may suggest Behçet disease. Oral ulceration can also occur in SLE, inflammatory bowel disease, infection, and drug toxicity.

Constitutional symptoms

Fever, weight loss, profound fatigue, and night sweats broaden the differential to systemic autoimmune disease, infection, malignancy, and autoinflammatory disease. Fever associated with an acutely inflamed joint must heighten concern for septic arthritis.

Medication and oncological history

Drug history has become increasingly relevant. Immune checkpoint inhibitors targeting CTLA-4, PD-1, PD-L1 and related pathways can induce inflammatory arthritis, polymyalgia-like syndromes, myositis, sicca manifestations, and vasculitis. Rheumatic immune-related adverse events may persist even after immunotherapy has been interrupted and require coordinated rheumatology–oncology management [5].

6. Rheumatoid Arthritis and the Spectrum of Pre-RA

RA remains the prototypical chronic inflammatory polyarthritis. The 2010 ACR/EULAR classification criteria represented a major shift toward recognition of earlier disease by integrating joint involvement, serology, acute-phase reactants, and symptom duration [6].

These criteria, however, are **classification criteria rather than diagnostic criteria**. Clinical diagnosis requires exclusion of competing explanations for synovitis.

6.1 Clinically suspect arthralgia

A particularly important development has been recognition of symptomatic stages preceding clinical arthritis.

The EULAR definition of arthralgia suspicious for progression to RA identified seven clinical features:

- symptom duration <1 year,
- MCP symptoms,
- morning stiffness ≥60 minutes,

- most severe symptoms in the early morning,
- first-degree relative with RA,
- difficulty making a fist,
- positive MCP squeeze test [7].

The field has advanced further with the 2025 EULAR/ACR risk-stratification criteria for individuals with arthralgia considered at risk of RA. In 2,293 symptomatic at-risk individuals, six clinical/serological variables—morning stiffness, patient-reported joint swelling, difficulty making a fist, CRP, rheumatoid factor (RF), and anti-citrullinated protein antibodies (ACPA)—provided meaningful prediction of inflammatory arthritis development. Addition of MRI-detected subclinical inflammation further improved discrimination [8].

This represents an important conceptual transition:

arthralgia → at-risk arthralgia → subclinical inflammation → clinically apparent inflammatory arthritis → established RA

Nevertheless, risk classification is not equivalent to an indication for routine preventive immunosuppression outside appropriate clinical contexts.

7. Laboratory Evaluation

Laboratory investigation should confirm or refine a clinically generated hypothesis rather than replace history and examination.

7.1 ESR and CRP

CRP and ESR are useful measures of systemic inflammation and contribute to disease activity assessment. However, neither test is sufficiently sensitive to exclude inflammatory arthritis.

Discordance may also occur. ESR is influenced by age, sex, anemia, immunoglobulin concentration, renal dysfunction, and other factors, whereas CRP generally changes more rapidly with acute inflammation.

7.2 Rheumatoid factor and ACPA

RF and ACPA are central investigations when RA is suspected.

ACPA generally provides greater specificity for RA and has prognostic significance for persistent and erosive disease. In early arthritis, both RF and ACPA contribute to prediction of persistent disease, with ACPA generally demonstrating greater diagnostic and prognostic utility [9].

Several principles are important:

- A negative RF does not exclude RA.
- Seronegative RA exists.

- Low-titer RF may occur in healthy older individuals and in chronic infection or other autoimmune diseases.
- ACPA positivity substantially increases the probability of RA when the clinical phenotype is compatible.
- Serology should not be ordered indiscriminately in patients with low pre-test probability.

7.3 Antinuclear antibodies

ANA testing should not be used as a general screening test for nonspecific musculoskeletal pain.

ANA should be requested when the clinical phenotype suggests SLE, systemic sclerosis, Sjögren syndrome, inflammatory myopathy, mixed connective tissue disease, or another ANA-associated condition. Subsequent testing should be guided by the ANA pattern and clinical manifestations.

The 2019 EULAR/ACR SLE classification criteria use a positive ANA at a titre of at least 1:80 as an entry criterion before weighted clinical and immunological domains are considered [10]. Again, these are classification rather than stand-alone diagnostic criteria.

7.4 HLA-B27

HLA-B27 testing is most useful when the pre-test probability of SpA is meaningful. It should not be used as a population screening test for nonspecific back pain.

A positive result supports SpA but does not establish the diagnosis, whereas a negative result does not exclude it.

8. Synovial Fluid Analysis: An Underused Diagnostic Tool

Arthrocentesis is among the highest-yield procedures in the assessment of unexplained acute inflammatory monoarthritis or oligoarthritis.

Synovial fluid should generally be assessed for:

- gross appearance,
- leukocyte count and differential,
- Gram stain,
- aerobic and anaerobic culture,
- crystal analysis using compensated polarized-light microscopy.

When infection is suspected, microbiological investigation takes priority.

8.1 Septic arthritis

Septic arthritis is a medical emergency because delayed treatment can rapidly result in cartilage destruction, systemic infection, and death.

No serum biomarker can reliably exclude septic arthritis. Synovial leukocyte count contributes to probability assessment but should not be interpreted as an absolute diagnostic threshold. Increasing synovial leukocyte counts increase the likelihood of infection, but crystal arthritis and other intense inflammatory states can also produce very high counts [11].

The SANJO guideline emphasizes aspiration of synovial fluid and microbiological identification of the pathogen as central components of diagnosis [12].

Importantly:

Identification of crystals does not exclude concomitant septic arthritis.

When clinical suspicion persists, synovial fluid culture remains necessary even when monosodium urate (MSU) or calcium pyrophosphate (CPP) crystals are identified.

9. Crystal-Induced Arthritis

9.1 Gout

Gout commonly presents as an abrupt, intensely painful monoarthritis, frequently involving the first MTP joint, ankle, or knee. Symptoms often reach maximal intensity within 24 hours.

The definitive demonstration of needle-shaped, negatively birefringent MSU crystals remains the reference standard for diagnosis. Hyperuricemia alone is insufficient because many hyperuricemic individuals never develop gout, while serum urate may fall during an acute attack [13].

Ultrasonography and dual-energy computed tomography (DECT) have become increasingly important. The double-contour sign and tophaceous deposits on US and urate deposition on DECT can strongly support the diagnosis.

The 2023 EULAR imaging recommendations state that US and DECT are recommended imaging modalities in the diagnostic assessment of gout and that characteristic MSU deposition on appropriate imaging can, in selected circumstances, establish the diagnosis without synovial fluid confirmation [14].

9.2 Calcium pyrophosphate deposition disease

CPPD should be considered particularly in older patients presenting with acute or chronic inflammatory arthritis involving the knees, wrists, shoulders, ankles, or elbows.

The 2023 ACR/EULAR CPPD classification criteria represented the first validated classification system for symptomatic CPPD disease. Crowned dens

syndrome or demonstration of CPP crystals in synovial fluid constitutes sufficient criteria in the appropriate clinical context; otherwise, weighted clinical, metabolic, laboratory, and imaging domains are applied [15].

Associated metabolic disorders—including hemochromatosis, hyperparathyroidism, hypomagnesemia, and hypophosphatasia—should particularly be considered in younger patients or atypical presentations.

10. Spondyloarthritis and Inflammatory Back Pain

Inflammatory joint pain may involve the axial skeleton, peripheral joints, entheses, or combinations of these sites.

Inflammatory back pain is characterized by features such as:

- onset at a young age,
- insidious onset,
- improvement with exercise,
- lack of improvement with rest,
- nocturnal pain, particularly during the second half of the night,
- alternating buttock pain.

The ASAS inflammatory back pain criteria provide a useful framework, but inflammatory back pain is neither sufficiently sensitive nor sufficiently specific to diagnose axial SpA independently [16].

The ASAS classification framework incorporates imaging and clinical pathways and recognizes both radiographic and non-radiographic axial SpA [17].

MRI of the sacroiliac joints is particularly valuable when axial SpA is suspected but radiographs are normal or equivocal. Bone marrow edema/osteitis compatible with active sacroiliitis supports the diagnosis, although MRI abnormalities must be interpreted cautiously because mechanically induced and postpartum bone marrow changes may mimic inflammatory sacroiliitis.

Current ASAS-EULAR recommendations emphasize integration of symptoms, objective inflammatory findings, imaging, extra-musculoskeletal manifestations, and treatment response rather than reliance on any single diagnostic variable [18].

11. The Expanding Role of Musculoskeletal Ultrasonography

Ultrasonography has become an extension of physical examination in modern rheumatology.

Gray-scale US can demonstrate synovial hypertrophy, effusion, erosions, tenosynovitis, enthesopathy, and crystal deposits. Power Doppler detects vascularity associated with active inflammation.

Its major advantages include:

- absence of ionizing radiation,
- dynamic assessment,
- bedside accessibility,
- ability to examine multiple joints,
- guidance of aspiration and injection,
- detection of subclinical inflammation.

However, subclinical imaging abnormalities are not synonymous with disease. Low-grade synovial hypertrophy and even Doppler abnormalities may occasionally occur outside classical inflammatory arthritis. Imaging must therefore answer a specific clinical question rather than substitute for clinical reasoning.

12. MRI: When Greater Sensitivity Matters

MRI can demonstrate synovitis, tenosynovitis, bone marrow edema/osteitis, erosions, enthesitis, and sacroiliitis before abnormalities become visible on conventional radiographs.

MRI is particularly useful in:

- suspected early axial SpA,
- diagnostically difficult early inflammatory arthritis,
- suspected occult synovitis or tenosynovitis,
- selected patients at high risk of RA,
- anatomically complex joints,
- evaluation of inflammatory versus structural pathology.

The emerging role of MRI in pre-RA is especially important. The 2025 EULAR/ACR risk-stratification study demonstrated that adding MRI-detected subclinical inflammation to clinical and serological variables improved prediction of progression from at-risk arthralgia to inflammatory arthritis [8].

Nevertheless, routine MRI for every patient with arthralgia is neither necessary nor appropriate.

Advanced quantitative MRI, high-resolution CT techniques, molecular imaging, and artificial-intelligence-assisted image analysis are under investigation as potential tools for precision phenotyping and prognostication [3].

13. Red Flags Requiring Immediate Action

Several presentations should interrupt the routine diagnostic algorithm.

Acute hot swollen joint

Septic arthritis must be considered until reasonably excluded.

Arthritis with systemic toxicity

Fever, hypotension, altered mental status, or marked inflammatory response requires urgent investigation for infection.

Immunosuppressed patient with new monoarthritis

Typical signs of infection may be attenuated.

Prosthetic joint pain and swelling

Prosthetic joint infection requires a distinct diagnostic pathway.

Acute arthritis after invasive procedure or bacteremia

Hematogenous or iatrogenic infection should be considered.

Arthritis with rapidly progressive neurological or vascular findings

Alternative emergencies—including spinal infection, vasculitis, compartment syndromes, or vascular occlusion—must be considered.

14. Differential Diagnosis According to Clinical Pattern

Clinical pattern	Major diagnostic considerations
Acute monoarthritis	Septic arthritis, gout, CPPD, trauma/hemarthrosis
Recurrent acute monoarthritis	Gout, CPPD, palindromic rheumatism
Symmetric small-joint polyarthritis	RA, SLE, viral arthritis, PsA
Asymmetric lower-limb oligoarthritis	SpA, reactive arthritis, PsA, IBD-associated arthritis
DIP arthritis	PsA, osteoarthritis, crystal disease
Arthritis + dactylitis	PsA, reactive arthritis
Arthritis + enthesitis	SpA spectrum
Arthritis + psoriasis/nail disease	PsA
Arthritis + uveitis	SpA, Behçet disease, sarcoidosis
Arthritis + diarrhea/IBD	Enteropathic arthritis, reactive arthritis
Arthritis + urethritis/cervicitis	Reactive arthritis

Arthritis + photosensitive rash/cytopenia	SLE
Arthritis + fever/rash/hyperferritinemia	Adult-onset Still disease, infection, malignancy
Arthritis after checkpoint inhibitor therapy	Immune-related inflammatory arthritis
Elderly patient with shoulder/hip girdle stiffness	Polymyalgia rheumatica; consider mimics

Table 2. Pattern-based differential diagnosis of inflammatory joint pain

15. A Practical Diagnostic Algorithm

A clinically useful approach can be summarized as follows.

Step 1 — Confirm the anatomical source

Determine whether symptoms arise from:

joint → tendon sheath → enthesis → bursa → muscle → bone → referred/non-musculoskeletal pain.

Step 2 — Establish whether inflammation is present

Search for:

swelling + warmth + prolonged stiffness + rest/night pain + functional restriction.

Use US when physical examination is equivocal and the result is likely to change management.

Step 3 — Define the pattern

Characterize:

acute/chronic → mono/oligo/polyarticular → symmetric/asymmetric → small/large joint → axial/peripheral → additive/migratory/intermittent.

Step 4 — Search for disease signatures

Specifically examine for:

psoriasis/nail disease, dactylitis, enthesitis, uveitis, IBD, preceding infection, Raynaud phenomenon, sicca, oral/genital ulcers, photosensitivity, fever, rash and constitutional symptoms.

Step 5 — Exclude urgent mimics

In acute monoarthritis:

infection must be actively excluded before assuming autoimmune or crystal arthritis.

Step 6 — Order hypothesis-driven laboratory tests

Basic tests may include:

CBC, CRP/ESR, renal and liver function and urinalysis.

Additional tests should be phenotype-driven:

RF/ACPA → suspected RA

ANA and disease-specific autoantibodies → suspected connective tissue disease

HLA-B27 → appropriately selected suspected SpA

serum urate → supportive information, not proof of gout

microbiology/viral testing → selected exposure or infection phenotype

Step 7 — Aspirate when appropriate

Especially:

acute unexplained monoarthritis, suspected infection, or suspected crystal disease.

Step 8 — Select imaging according to the clinical question

Radiography: structural damage and baseline evaluation.

Ultrasound: synovitis, tenosynovitis, enthesitis, effusion, crystals, aspiration guidance.

MRI: occult/subclinical inflammation, sacroiliitis, bone marrow edema, complex diagnostic uncertainty.

DECT: selected cases of suspected urate deposition.

Step 9 — Reassess over time

A single encounter may not establish a definitive diagnosis. Undifferentiated inflammatory arthritis should be followed longitudinally because the phenotype may evolve into RA, PsA, SpA, connective tissue disease, or occasionally resolve spontaneously.

16. From Diagnosis to Early Treatment

The purpose of early diagnosis is not merely to assign a disease label but to prevent irreversible damage and disability.

For patients with persistent inflammatory arthritis, delayed rheumatologic assessment and delayed DMARD therapy are associated with poorer outcomes. The EULAR early arthritis recommendations therefore support rapid specialist evaluation and early treatment of patients at high risk of persistent disease [1].

For established RA, the most recent EULAR recommendations continue to support immediate DMARD initiation after diagnosis, methotrexate-based initial

therapy in most patients, regular disease activity assessment, and treatment adjustment until remission or low disease activity is achieved [2].

Importantly, treatment should not precede adequate exclusion of infection when the clinical presentation is compatible with septic arthritis.

Similarly, symptomatic therapy with glucocorticoids before diagnostic aspiration can obscure infection and should be avoided in an unexplained acute hot joint until infection has been appropriately evaluated.

17. Emerging Concepts and Future Directions

The approach to inflammatory joint pain is increasingly moving from **disease recognition after clinical arthritis develops** toward **risk identification before irreversible disease becomes established**.

RA provides the clearest example. Autoimmunity may precede clinical arthritis by years, followed by symptomatic arthralgia, subclinical imaging abnormalities, and eventually clinically apparent synovitis. The recent EULAR/ACR risk-stratification criteria represent a major step toward defining reproducible risk states [8].

A parallel evolution is occurring in PsA, where psoriasis, arthralgia, imaging abnormalities, and early musculoskeletal symptoms are increasingly conceptualized as stages preceding established PsA.

Advanced imaging is also evolving. High-resolution peripheral quantitative CT, novel MRI sequences, molecular imaging, optical techniques, and artificial intelligence may ultimately permit more precise characterization of inflammatory phenotypes [3]. At present, however, their routine diagnostic role remains limited.

The future diagnostic model will probably integrate:

clinical phenotype + autoantibody profile + imaging phenotype + genetics + molecular biomarkers + longitudinal data.

Until validated precision tools become available, careful clinical pattern recognition remains the foundation of diagnosis.

18. Conclusion

Inflammatory joint pain should be approached as a clinical syndrome rather than as a single disease entity. The most effective diagnostic strategy begins with confirmation of true articular inflammation and proceeds through systematic assessment of temporal course, joint distribution, symmetry, axial involvement, enthesitis, dactylitis, and extra-articular manifestations.

Laboratory biomarkers and imaging substantially refine diagnostic probability but should not replace clinical reasoning. RF and ACPA are most useful when

RA is clinically plausible; ANA testing should be driven by features of systemic autoimmune disease; and HLA-B27 should be interpreted within an appropriate SpA phenotype. Ultrasonography and MRI have improved detection of subclinical inflammation, while DECT and ultrasonography increasingly support recognition of crystal deposition.

Acute monoarthritis deserves special attention because septic arthritis must be rapidly excluded, regardless of the presence of crystals or a previous rheumatic diagnosis. Conversely, persistent small-joint inflammatory symptoms require early recognition because contemporary evidence supports a continuum extending from at-risk arthralgia to subclinical inflammation and clinically established RA.

The modern approach can therefore be summarized in one principle: **identify inflammation, recognize the pattern, exclude dangerous mimics, use targeted investigations to modify diagnostic probability, and reassess the phenotype longitudinally.** This approach maximizes diagnostic accuracy while minimizing indiscriminate serological testing, unnecessary imaging, and delays in disease-modifying therapy.

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CHAPTER 14

Artificial Intelligence and Digital Technologies in Periodontology Education: Current Applications, Evidence, and Future Directions

***Metehan Ozdemir*[1]**
***Yunus Emre Balaban*[2]**

[1] MSc, Selcuk University, Faculty of Dentistry, Department of Periodontology, Konya, Turkey ORCID: 0000-0002-5887-5665
[2] MSc, Asst. Prof. Selcuk University, Faculty of Dentistry, Department of Periodontology, Konya, Turkey ORCID: 0000-0002-1754-4296

1. Introduction

Artificial intelligence (AI) has become increasingly used in health sciences and dentistry in recent years. In particular, advances in generative AI systems with natural language processing capabilities and large language models (LLMs) have substantially expanded the use of these technologies in education. Systems such as ChatGPT, which can interact with users through natural language, are considered alternative learning tools for students because of their ability to provide rapid access to information, explain complex topics, generate educational content, and offer personalized feedback (El-Hakim, Anthonappa, & Fawzy, 2025; Reyes-Alardo et al., 2026).

Beyond text-based generative AI, other AI approaches, including machine learning and deep learning, have also been applied in dental education. Convolutional neural networks (CNNs), a type of deep learning architecture particularly suited to image analysis, have been used to support the interpretation of dental and periodontal radiographic images (Glick, Clayton, Angelov, & Chang, 2022; Wei et al., 2026).

Dental education is a multidimensional process that requires the development of preclinical skills, clinical decision-making, patient communication, and professional competencies alongside theoretical knowledge. Therefore, the applications of AI in dental education are not limited to access to information but also include areas such as preclinical and clinical training, diagnostic assessment, student feedback, assessment and evaluation, and the development of educational content. Recent reviews suggest that AI-supported applications may contribute to educational outcomes such as knowledge acquisition, technical skills, and clinical decision-making. However, AI should be used as a complementary tool rather than as a replacement for traditional educational methods (El-Hakim et al., 2025; Reyes-Alardo et al., 2026).

In periodontology education, digital approaches began to be used through virtual classroom and virtual patient systems before the emergence of generative AI applications. These approaches have been reported to support problem-based learning and contribute to the development of students' history-taking skills before their interactions with real patients (Mattheos, Nattestad, Schittek, & Attström, 2001; Schittek Janda et al., 2004). More recently, virtual reality (VR), which provides computer-generated interactive simulated environments, and haptics-based simulation have been used in periodontal training, particularly for developing practical skills such as periodontal probing, scaling, and root planing (Cheng, Hao, Lin, Liu, & Yu, 2025; Wei et al., 2026; Zhang et al., 2021).

However, the use of AI in education also raises several concerns, including accuracy and reliability, overreliance, academic integrity, and ethical use. Therefore, it is important for students to critically evaluate AI-generated information and to use these systems as tools that support, rather than replace,

independent thinking and clinical reasoning (El-Hakim et al., 2025; Knight, Parthasarathy, & Huang, 2026).

This chapter aims to evaluate the current applications of artificial intelligence and related digital technologies in periodontology education based on the available literature. In this context, the chapter will address theoretical education and access to information, case-based learning and clinical decision-making, preclinical education and simulation, student feedback and assessment, as well as the educational limitations of AI and approaches to its future integration into periodontology curricula.

2. Current Applications in Periodontology Education

2.1. Theoretical Education and Access to Information

AI-based tools enable students to access information rapidly and interactively, explore concepts through questioning, and receive personalized feedback. Adaptive learning systems, intelligent tutoring tools, and LLMs have been reported to support conceptual learning, knowledge retention, and student engagement (Reyes-Alardo et al., 2026).

In a study conducted among dental students, Khurana et al. reported that 80.7% of participants used LLMs, with explaining concepts (59%) and exam preparation (36%) among the most common purposes of use. In addition, 62% of students stated that these tools helped them save time. Despite these perceived benefits, participants also expressed concerns regarding the accuracy of AI-generated information, discipline-specific limitations, and ethical issues (Khurana et al., 2026).

Studies in periodontology also indicate that the potential of LLMs in theoretical education may vary depending on the model used. Danesh et al. reported that ChatGPT-4 achieved an accuracy of 73.6% on questions at the level of postgraduate periodontology education, compared with 57.9% for ChatGPT-3.5 (Danesh, Pazouki, Danesh, Danesh, & Vardar-Sengul, 2024). Similarly, C. Li et al., who evaluated performance on periodontal surgery topics, found that ChatGPT-4 performed better than the earlier model but remained below the overall performance of dental students (C. Li, Zhang, Abdul-Masih, Zhang, & Yang, 2025).

In a study comparing AI-assisted learning with traditional educational materials, both ChatGPT-4o and written materials improved students' knowledge of periodontal-systemic relationships, with no significant difference between the two methods in terms of short-term knowledge acquisition. ChatGPT-4o was rated more positively for access to information and motivation, whereas written materials provided an advantage in terms of concentration (Keles, 2026). These findings suggest that AI-assisted learning may serve as a complementary

approach to traditional educational methods rather than as a replacement for them.

2.2. Case-Based Learning and Clinical Decision-Making

In periodontology education, the assessment of periodontal findings, disease classification, and appropriate clinical decision-making are important areas of learning. Large language models have been suggested as supportive educational tools for applying periodontal classification systems and assisting clinical decision-making processes (Ji et al., 2026).

One of these applications is the classification of periodontitis. Tastan Eroglu et al. reported that ChatGPT achieved an accuracy of 84.0% in determining disease extent, 59.5% in staging, and 50.5% in grading. While the model performed better in determining disease extent, its performance was lower particularly in grading, and some incorrect classifications were presented with a high degree of confidence (Tastan Eroglu, Babayigit, Ozkan Sen, & Ucan Yarkac, 2024).

Ji et al. compared the performance of ChatGPT-4 in periodontal classification with that of dental educators and reported an overall accuracy of 75% for educators and 63.3% for ChatGPT-4. Although there was no statistically significant difference between the overall performance of the educators and ChatGPT-4, some educators achieved higher performance than the model (Ji et al., 2026).

The use of AI in case-based learning is not limited to the classification of periodontitis. In their evaluation of endo-periodontal lesions, Baris and Baris reported accuracy rates of 90.8% for Scholar GPT, 89.8% for ChatGPT-4o, and 78.1% for Scholar AI. Due to differences in performance among the models and the possibility of incorrect responses, the authors emphasized the need to verify information provided by AI (Baris & Baris, 2026).

Taken together, these findings suggest that LLMs may serve as supportive educational tools in case-based learning and clinical decision-making. However, variations in performance across different diagnostic tasks and AI models, together with the possibility of incorrect outputs, highlight the importance of verifying AI-generated information and using these systems under appropriate educational guidance.

2.3. Preclinical Education and Simulation

In preclinical education, simulation systems provide controlled and repeatable learning environments in which students can develop their technical and communication skills before clinical practice. In periodontology education, virtual reality (VR) and virtual patient applications are used alongside traditional dental models (Schittek Janda et al., 2004; Zhang et al., 2021).

Zhang et al., who evaluated the combined use of virtual and traditional methods in periodontal skills training, reported that the combination of VR and dental models improved students' theoretical knowledge and practical performance. They also found that using VR after training with dental models was particularly effective (Zhang et al., 2021). Similarly, Cheng et al. demonstrated that VR and traditional models provided advantages for different skills in scaling and root planing training, with the highest overall performance observed in the group in which both methods were combined (Cheng et al., 2025).

Simulation-based applications can also be used to develop communication and patient assessment skills. Schittek Janda et al. reported that students who practiced history-taking with a virtual patient asked more relevant questions and obtained more comprehensive patient histories during subsequent interactions with real patients (Schittek Janda et al., 2004).

2.4. Artificial Intelligence in Student Assessment and Feedback

AI-based systems can support the evaluation of student responses and the provision of feedback. In periodontal surgery education, ChatGPT-4 evaluations of student responses were found to be consistent with those of faculty members (C. Li et al., 2025). In preclinical periodontology education, M.-H. Li et al. combined peer role-play with AI-assisted feedback, complemented by peer assessment and faculty supervision. The feedback addressed professional accuracy, patient-friendly communication, empathy, and overall communication skills. In that study, 86% of students considered the approach valuable for learning, while 75% found it useful for developing communication skills. The approach also provided students with an opportunity to practice explaining periodontal information in a way that patients could understand (M.-H. Li et al., 2026).

In periodontology, Ma et al. compared an AI-generated examination with a traditional examination prepared by faculty members. The AI-generated examination was reported to have greater curriculum coverage, while both examinations showed similar reliability and difficulty characteristics. However, the AI-generated questions showed a lower ability to distinguish between students with different levels of performance. In addition, students rated the traditional examination more positively in terms of appropriate difficulty, knowledge coverage, and support for learning (Ma, Pan, & Yu, 2025).

3. Artificial Intelligence Literacy, Ethics, and Educational Limitations

The increasing use of AI tools has made it important for students to develop sufficient AI literacy to understand both the capabilities and limitations of these technologies. Altındağ et al. reported that more than half of dental students and dentists had only a basic understanding of AI, while the proportion who had received information about AI as part of their university education was relatively

low. In contrast, more than 70% of participants supported the integration of AI into dental education to support diagnostic reasoning and learning processes (Altındağ, Uzun, Altındağ, & Orhan, 2026).

Students' attitudes toward AI should also be considered when integrating these technologies into dental education. AI literacy involves understanding not only how to use these tools but also their capabilities, limitations, and appropriate areas of use. AI-related anxiety among dental students has been reported to be moderate and associated with factors such as academic seniority and duration of AI use (Karaaslan & Erişken, 2026).

One of the main limitations of AI use is the accuracy and reliability of the information it generates. Although dental students viewed the time-saving benefits of LLMs positively, they also expressed concerns about information accuracy, limitations specific to dentistry, and ethical issues (Khurana et al., 2026). Critically evaluating AI-generated information and, when necessary, comparing it with reliable scientific sources are important for maintaining students' independent thinking and clinical reasoning skills (Knight et al., 2026).

The use of AI in dental education also raises ethical issues related to patient data privacy, academic integrity, algorithmic bias, and the reliability of generated content. Therefore, educational programs should establish clear guidelines for AI use, improve faculty competence in this area, and guide students toward the ethical and responsible use of AI (El-Hakim et al., 2025).

4. The Future of Artificial Intelligence in Periodontology Education

The increasing use of AI in dental education has highlighted the need for its structured integration into educational programs. Although structured AI education within the curriculum remains limited among dental students and dentists, positive attitudes toward the integration of AI into dental education suggest that AI literacy may receive greater emphasis in future curricula (Altındağ et al., 2026).

One potential future application of AI in periodontology education is the development of learning and feedback systems that can be adapted to individual student needs. Identifying students' needs in theoretical knowledge, case assessment, and preclinical practice and providing personalized feedback are considered among the potential applications of AI in education (El-Hakim et al., 2025).

The use of AI in reflective learning and the evaluation of clinical experiences may also become increasingly important in the future. However, these systems should be used to support the educational process without replacing students' independent thinking, self-assessment, and clinical reasoning (Knight et al., 2026).

5. Conclusion

Artificial intelligence and digital technologies have the potential to support various areas of periodontology education, including theoretical learning, case-based clinical decision-making, preclinical training and simulation, student assessment, and feedback. Current studies suggest that AI-assisted applications can support access to information and learning processes while providing students with different opportunities for education and feedback. However, differences in performance among AI models, the accuracy of generated information, and limitations in clinical reasoning should be taken into consideration.

Rather than replacing traditional educational methods, AI appears to be more appropriately used as a complementary tool that supports learning and clinical reasoning under faculty guidance. Developing AI literacy, critically evaluating AI-generated information, and incorporating principles of ethical AI use into the educational process are important for the effective and responsible integration of AI into periodontology education.

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CHAPTER 15

Biomimetic Approaches in Indirect Posterior Restorations: New-Generation Restorative Materials and Surface Adhesion Protocols

Sedef Kurt Çıralık[1]

[1] Arş. Gör, Recep Tayyip Erdoğan Üniversitesi Diş Hekimliği Fakültesi, ORCID iD:0009-0004-7454-5369

1.Biomimetic Dentistry Philosophy

For many years, the conventional approach to prosthodontics was based on macro-mechanical preparation principles centered on retention and resistance form. Although this approach may ensure the retention of restorations in the oral cavity, it may also result in excessive loss of healthy dental tissue and disruption of the tooth's biomechanical balance (1). The biomimetic dentistry philosophy that has emerged in modern prosthodontics is based on the principle of "imitating nature." Its primary goal is to preserve pulp vitality and restore lost hard tissues in a tissue-friendly manner using materials that most closely approximate the original anatomical and mechanical properties of the tooth, including elastic modulus, strength, and light transmission (1,2). Three fundamental stages of a biomimetic approach in the posterior region can be identified (1,3):

Maximum preservation of hard tissues: Preparation margins should be determined according to the anatomical boundaries of the defect rather than according to predetermined preparation angles.

Optimization of stress distribution: The natural biomechanical balance between rigid enamel and more compliant dentin should be approximated.

Preservation of hybrid-layer integrity: Advanced surface pretreatment protocols should be used to support the long-term performance of the adhesive interface.

2. Types of Indirect Restorations in the Posterior Region and Clinical Decision-Making

In posterior teeth with extensive loss of tooth structure, direct composite restorations may be susceptible to failure because of polymerization shrinkage, marginal leakage, and inadequate occlusal contacts (2). Indirect and semi-direct techniques may overcome some of these limitations.

2.1. Indirect Restoration Geometries

The selection of indirect restoration geometries for managing hard-tissue loss in posterior teeth depends on cavity depth, the amount of remaining tooth structure, and the degree of cuspal support. Inlays are generally indicated in limited cases without cuspal loss and with sufficiently supported cavity walls; however, their indications have become more restricted within a biomimetic treatment philosophy (2). In contrast, when at least one cusp is weakened or fractured, onlay restorations incorporating cuspal coverage may be preferred to facilitate a more favorable distribution of occlusal loads (3). When the loss of tooth structure is more extensive and the entire occlusal table needs to be covered while protecting the remaining cusps, overlay restorations may be considered as a minimally invasive design (3). Finally, in extensively damaged endodontically treated posterior teeth, endocrowns have emerged as monolithic restorative

options that use the depth and retention of the pulp chamber and may eliminate the need for conventional post-and-core procedures (4).

2.2. Deep Margin Elevation (DME)

In the presence of subgingival margins, digital or conventional impression procedures, isolation, and cementation become considerably more challenging. The DME technique involves relocating a subgingival cervical margin to a supragingival position using a matrix band and composite resin (5,6). DME may facilitate isolation and restorative procedures in cases with deep cervical margins; however, its long-term periodontal and adhesive benefits should be interpreted in light of the available evidence.

3. New-Generation Restorative Materials and Biomimetic Comparison

Material selection for indirect posterior restorations should consider resistance to masticatory forces, wear of the opposing dentition, and an elastic modulus compatible with tooth tissues (7).

3.1. Glass Ceramics

• Lithium disilicate ceramics: With high flexural strength (approximately 350–500 MPa) and favorable esthetic and adhesive characteristics, lithium disilicate is widely used for indirect posterior restorations (3,7).

• Zirconia-reinforced lithium silicates (ZLS): These glass-ceramic materials contain zirconium oxide and are characterized by a fine crystalline microstructure, offering favorable processing characteristics and surface quality (7).

3.2. Resin-Matrix Ceramics and Hybrid Blocks

• Polymer-infiltrated ceramic networks (PICNs): These materials are produced by infiltrating a porous ceramic network with a polymer matrix (e.g., VITA Enamic) (9).

• Resin nanoceramics (RNCs): These are CAD/CAM materials containing a high proportion of nano-sized fillers (9).

Biomimetic advantage: The elastic modulus of some resin-matrix ceramic and hybrid materials (approximately 15–20 GPa) is relatively close to that of human dentin (approximately 18 GPa). This similarity may contribute to stress attenuation and may influence stress transfer to the underlying tooth tissues (7,9).

3.3. Translucent Monolithic Zirconia

New-generation 4Y-PSZ and 5Y-PSZ zirconia materials, with increased cubic-phase content, combine high strength with improved optical properties (8,10). They may be considered in patients with parafunctional habits and in cases

requiring conservative reduction, such as selected overlay or endocrown restorations (4,8).

4. Surface Pretreatment and Adhesion Protocols

The success of adhesive dentistry depends substantially on appropriate conditioning and treatment of both the tooth–adhesive interface and the internal surface of the restoration (13).

4.1. Tooth-Surface Protocols and Immediate Dentin Sealing (IDS)

Immediate dentin sealing (IDS) is an important component of biomimetic adhesive restorative protocols (1,11,12).

Clinical advantages of IDS:

• Immediate hybridization of freshly cut dentinal tubules may reduce postoperative sensitivity (11).

• The adhesive interface can mature before exposure to provisional-restoration materials and oral contamination (1,12).

• Immediate sealing has been associated with higher bond-strength values than delayed dentin sealing in many in-vitro investigations (12).

4.2. Restoration Surface Pretreatment Protocols

The recommended surface-conditioning protocol varies according to the chemical composition and micromechanical characteristics of the restorative material (13,14).

A. Glass ceramics (lithium disilicate and ZLS)

1. Hydrofluoric acid (HF) etching: HF is used to etch the internal surface of glass ceramics. The exact concentration and etching time should follow the manufacturer's instructions because they vary according to the ceramic composition (7,14).

2. Cleaning after etching: After HF etching, the surface should be thoroughly rinsed and cleaned to remove reaction products and residues before silanization.

3. Silanization: A suitable silane coupling agent is applied according to the manufacturer's instructions. Silane promotes chemical coupling between the silica-containing ceramic surface and the organic resin cement (13,14).

B. Resin-matrix ceramics and hybrid blocks

1. Air abrasion: Controlled air abrasion with alumina particles may be used according to the specific CAD/CAM material and manufacturer's recommendations. Excessive air-abrasion pressure may damage resin-based CAD/CAM blocks (9,16).

2. Cleaning and silanization/adhesive application: Following surface cleaning, a silane-containing agent or an adhesive system containing an appropriate functional monomer such as 10-MDP may be selected according to the restorative material and manufacturer's instructions (13,16).

C. Zirconia ceramics (APC concept)

Because zirconia does not contain a glass phase, hydrofluoric acid etching is not an effective surface-conditioning method. An air-abrasion–primer–composite (APC) concept is commonly recommended (15).

Air-abrasion: Controlled air abrasion with alumina or silica-coated alumina particles may be used at a pressure appropriate for the zirconia system (15).

Primer: A zirconia primer or universal adhesive containing 10-MDP may be applied. Functional phosphate monomers can chemically interact with zirconia oxide surfaces (13,15).

Composite resin: A compatible resin cement, commonly a dual-cure resin cement when indicated, is used for definitive cementation (15,17).

5. Adhesive Cementation Protocol and Clinical Tips

Adhesive cementation is a critical final stage that facilitates load transfer between the restoration and the tooth (17).

5.1. Cement selection:

• Dual-cure resin cements: In indirect restorations where light transmission is limited, dual-cure resin cements may be preferred because they combine light- and chemical-curing mechanisms (17).

• Preheated composite resin: For thin or highly translucent indirect restorations such as onlays and overlays, preheating composite resin can reduce viscosity and facilitate seating. The literature reports a range of preheating temperatures, with approximately 54–68°C among the most frequently reported values; protocols should be standardized according to the material and clinical system used (18).

6. Clinical Complications and Management of Failures

Although indirect posterior restorations generally demonstrate favorable clinical performance and survival, complications may arise from material selection, preparation geometry, occlusal load distribution, or technical deficiencies during adhesive cementation (19). Accurate diagnosis and classification of complications, followed by minimally invasive management consistent with biomimetic principles, are important for preserving tooth structure and extending restoration longevity (19,20).

6.1. Adhesive Failures and Loss of Bond (Debonding)

Debonding is a mechanical/biological complication characterized by loss of retention of the restoration and/or marginal defects. It may be associated with contamination by saliva or blood during cementation, inappropriate conditioning of the restoration's internal surface, inadequate silanization, or insufficient control of polymerization-related stresses (20).

When IDS is not performed immediately after dentin preparation, the exposed dentin may become contaminated during the provisional phase, potentially compromising the adhesive interface (11,12). If debonding occurs while the restoration remains intact and marginal adaptation is acceptable, the restoration and tooth surfaces may be reconditioned and the adhesive cementation protocol repeated when clinically appropriate (20).

6.2. Ceramic and Material Fractures (Chipping vs. Catastrophic Fracture)

Mechanical fractures in indirect posterior restorations can broadly be considered as localized chipping or more extensive structural fractures (21).

1. Chipping: Localized fracture may occur when ceramic or hybrid materials are subjected to stresses that exceed their local tolerance, when occlusal adjustment is inadequate, or when restoration thickness is insufficient. If overall restoration integrity and marginal adaptation are maintained, intraoral repair may be considered rather than complete replacement (21,22).

2. Catastrophic structural fractures: These involve extensive fracture of the restoration and/or underlying tooth structure. Risk may increase in patients with parafunctional habits, inappropriate material selection, inadequate cuspal coverage, or pre-existing deep cracks (21). In extensive fractures, the restoration should be removed and the remaining tooth structure reassessed biomechanically; an overlay or endocrown design may be considered when indicated (4,21).

6.3. Biological Complications: Secondary Caries and Pulpal Reactions

Secondary caries may be associated with marginal leakage, inadequate finishing and polishing, inappropriate margin design, and plaque accumulation around subgingival margins (23). In the presence of subgingival margins, inadequate execution of DME may also adversely affect periodontal conditions (5,6).

Failure to seal exposed dentin before cementation or failure to identify premature occlusal contacts after cementation may contribute to postoperative sensitivity and pulpal irritation (1,23). When secondary caries develops, localized repair or correction of the cervical margin may be considered where appropriate. If pulpal involvement occurs, endodontic treatment may be performed through an appropriate access cavity without necessarily removing an otherwise serviceable

restoration; the access cavity should subsequently be sealed with an appropriate restorative material (23).

6.4. Intraoral Ceramic Repair Protocol

For glass-ceramic, zirconia, or hybrid restorations exhibiting chipping while maintaining acceptable marginal integrity, an intraoral repair approach may help preserve tooth structure and avoid complete replacement (22,24).

1. Isolation and surface preparation: Isolate the area with a rubber dam. Roughen the fractured restoration surface and exposed tooth structure using an appropriate abrasive system or fine-grit diamond instruments.

2. Chemical surface activation:

• Glass-ceramic surfaces: Etch with hydrofluoric acid according to the manufacturer's recommended concentration and duration, then rinse and dry.

• Zirconia surfaces: After air abrasion, apply a zirconia primer or adhesive containing 10-MDP.

• Exposed tooth structure: Etch enamel and dentin with phosphoric acid according to the selected adhesive system's instructions.

3. Silanization and adhesive application: Apply a suitable silane agent to glass-ceramic surfaces when indicated, followed by the appropriate adhesive system and polymerization.

4. Restoration and polishing: Restore the fractured area incrementally with a suitable nano-hybrid or microfilled composite resin and finish/polish the repaired area (22,24).

Conclusion

In indirect posterior restorations, the biomimetic approach is not merely an esthetic preference; rather, it aims to preserve the tooth as closely as possible to its natural biomechanical and functional state. To achieve successful biomimetic restorative treatment:

1. Unnecessary loss of tooth structure should be avoided, and decisions regarding cuspal preservation and coverage should be based on biomechanical considerations (1,3).

2. Dentin should be sealed immediately after preparation when IDS is indicated (11,12).

3. Material selection should consider not only esthetics and fracture resistance but also compatibility of elastic modulus with tooth tissues (7,9).

4. Material-specific surface-conditioning protocols (e.g., HF/silane for glass ceramics or air abrasion/MDP for zirconia) should be followed according to current evidence and manufacturer recommendations (13–15).

In the future, digital scanning and artificial-intelligence-assisted CAD/CAM systems may facilitate the production of gradient or functionally graded restorative materials designed to reproduce the natural stress distribution of teeth, potentially advancing biomimetic dentistry.

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CHAPTER 16

Periodontal Splinting in Restorative Dentistry: Concepts And Applications

Emre Taslı[1]
Metehan Ozdemir[2]
E. Elif Mutafcılar Velioglu[3]
Mehmet Semih Velioglu[4]

[1] MSc, Selcuk University, Faculty of Dentistry, Department of Periodontology, Konya, Turkey, ORCID: 0000-0002-2341-7058
[2] MSc, Selcuk University, Faculty of Dentistry, Department of Periodontology, Konya, Turkey, ORCID: 0000-0002-5887-5665
[3] MSc, Asst. Prof., Selcuk University, Faculty of Dentistry, Department of Periodontology, Konya, Turkey, ORCID: 0000-0003-1882-553X
[4] MSc, Asst. Prof., Selcuk University, Faculty of Dentistry, Department of Restorative Dentistry, Konya, Turkey, ORCID: 0000-0001-8541-6483

1. INTRODUCTION

Periodontal diseases are multifactorial, chronic inflammatory conditions that affect the gingiva, cementum, periodontal ligament, and alveolar bone, which surround and support the teeth within the jaws. These diseases may lead to progressive attachment loss and, ultimately, tooth loss (Kassebaum et al., 2014; Sanz et al., 2020). This process, initiated by the microbial dental biofilm and shaped by the host immune-inflammatory response, may manifest as reversible gingivitis, in which inflammation is confined to the marginal gingiva, or as periodontitis, characterized by irreversible destruction of the deeper supporting tissues (Armitage, 1999; Chapple et al., 2018).

As periodontitis progresses, connective tissue attachment loss and alveolar bone resorption reduce periodontal support, and finite element analyses have shown that the tooth's center of resistance shifts toward the apex as alveolar bone is loss (Geramy, 2000). As a result, the length of root supported by the surrounding bone decreases relative to the clinical crown, which increases the lever arm of occlusal forces. Under the same load, teeth with reduced bone support show greater displacement and higher stresses in the periodontal ligament, which may increase their tendency for lateral displacement (Geramy, 2000; Ona & Wakabayashi, 2006).

In a healthy periodontium, the elastic properties of the periodontal ligament allow a certain degree of physiological tooth mobility within normal limits. However, as periodontal tissue destruction progresses and the center of resistance shifts apically, physiological mobility may develop into pathological mobility. Pathological mobility is of considerable clinical importance, as it may contribute to progressive tooth mobility associated with secondary occlusal trauma in teeth with reduced periodontal support and may directly impair masticatory efficiency, biting force, phonetics, and esthetic appearance (Kathariya et al., 2016; Newman, Takei, Klokkevold, & Carranza, 2011).

2. DEFINITION AND BIOMECHANICAL OBJECTIVES OF SPLINTING

In dentistry, splinting is defined as the stabilization of teeth exhibiting pathological mobility due to loss of supporting tissues caused by periodontal destruction, dental trauma, or various surgical procedures. This is achieved by connecting the affected teeth to more stable adjacent teeth within the dental arch to form a single functional unit, thereby helping to maintain masticatory function and physiological tooth position (Aslan & Acun Kaya, 2022). The primary principle of periodontal splinting is to limit abnormal three-dimensional mobility of the compromised tooth and to biomechanically redistribute the destructive occlusal forces acting on it (Kathariya et al., 2016).

From a biomechanical perspective, a key rationale for splinting is the distribution of occlusal forces over a larger periodontal surface area; finite element analyses of periodontally compromised mandibular anterior teeth have shown that splints of different materials distribute occlusal loads among the splinted teeth and reduce stresses in the periodontal tissues of the compromised teeth, particularly as bone support decreases (Kurgan, Terzioglu, & Yılmaz, 2014; Liu et al., 2022). During mastication and perioral muscle activity, the resulting forces are transferred to the more stable teeth included in the splint rather than being concentrated on a single tooth with reduced periodontal support (Kathariya et al., 2016; Kurgan et al., 2014). By redistributing occlusal loads among the teeth included in the splint, splinting may reduce both the load and mobility of the affected tooth (Aslan & Acun Kaya, 2022; Kathariya et al., 2016).

In addition, following periodontal surgery and regenerative procedures, the mechanical stabilization provided by splinting is intended to minimize micromovements of the surgical site that may interfere with healing (Sharma, 2017; Strassler, Tomona, & Spitznagel, 2003). Wound stability, including stable adhesion of the fibrin clot to the root surface, is considered a prerequisite for periodontal regeneration, and this fibrin organization may be disturbed in mobile teeth (Gürses & Öncü, 2019; Susin et al., 2015).

3. CLINICAL INDICATIONS AND CONTRAINDICATIONS OF PERIODONTAL SPLINTING

The primary clinical indication for periodontal splinting is persistent or progressive pathological tooth mobility associated with secondary occlusal trauma despite the completion of initial periodontal therapy and occlusal adjustment (Dommisch et al., 2022; Kathariya et al., 2016). Splinting may be considered particularly for teeth with Miller grade II or III mobility (Miller, 1950; Sood & Kaur, 2015), as well as teeth with grade I mobility that do not respond adequately to initial therapy, when mobility adversely affects the patient's masticatory function, biting force, and comfort (Aslan & Acun Kaya, 2022; Kathariya et al., 2016). According to Lindhe's clinical decision-making model, stabilization may be considered as part of the treatment plan, particularly for teeth with increased mobility due to advanced loss of periodontal support when the mobility causes functional discomfort (Aslan & Acun Kaya, 2022; Lang & Lindhe, 2015). Additional indications for periodontal splinting include stabilization of mobile teeth during and after regenerative periodontal surgery to minimize micromovements that may interfere with wound healing (Gupta, Tomer, & Parvathi Jayan, 2023; Strassler et al., 2003), acute stabilization in cases of dental trauma (Kahler, Hu, Marriot-Smith, & Heithersay, 2016), and a possible improvement in patients' oral health-related quality of life (OHRQoL) (Sonnenschein et al., 2021).

In contrast, periodontal splinting has certain contraindications and limitations, as inappropriate case selection or failure to follow biological principles may further aggravate existing periodontal tissue destruction. Inadequate plaque control is an important limiting factor, particularly in patients with poor oral hygiene or limited compliance with oral hygiene measures, because the splint may create additional plaque-retentive areas and contribute to periodontal inflammation. Other clinical factors that should be considered when deciding on splinting include uncontrolled active periodontal inflammation, lack of appropriate occlusal adjustment, teeth with a hopeless prognosis, high caries activity, and severe crowding or occlusal discrepancies that may compromise the functional adaptation of the splint (Bhuvaneswari, Gowri, Ram Kumar, & Vanitha, 2019; Kathariya et al., 2016).

4. ADVANTAGES AND DISADVANTAGES OF PERIODONTAL SPLINTING

As described in Section 2, the primary clinical advantage of periodontal splinting is the stabilization of compromised teeth with pathological mobility, which may reduce both the load and mobility of the affected tooth (Aslan & Acun Kaya, 2022). Splinting has been proposed to improve wound stability following regenerative and surgical periodontal procedures by reducing tooth movement. (Strassler et al., 2003; Susin et al., 2015); however, in a propensity score-matched analysis of intrabony defects treated with enamel matrix derivative in teeth with mobility degree 0 or 1, splinting provided no statistically significant additional improvement in clinical attachment level, probing depth, or radiographic bone defect depth over 3 years (Mikami et al., 2025). In addition, splinting may reduce patient discomfort and improve masticatory function (Strassler & Brown, 2001; Su & Cai, 2018), and in a pilot randomized clinical trial, splinting mobile mandibular incisors showed a non-significant tendency toward greater improvement in OHRQoL (Sonnenschein et al., 2021).

Despite these important advantages, periodontal splinting also has certain clinical and restorative disadvantages that should be considered. If the embrasure areas are not properly designed and adequate spaces for oral hygiene are not provided, plaque accumulation may occur, potentially leading to recurrent gingival inflammation and increased periodontal probing depths (Kathariya et al., 2016). From a biomechanical perspective, repeated exposure to masticatory loads in the moist oral environment may lead to composite resin wear, deterioration of marginal adaptation, or debonding of the splint over time (Zhang et al., 2023). In addition, areas that are difficult to maintain may be associated with an increased risk of secondary caries over time, while excessively bulky restorations may compromise esthetics and phonetics (Eley, Soory, & Manson, 2010; Hussain, 2020).

5. CLASSIFICATION OF PERIODONTAL SPLINTS

To establish standardized terminology, various classification systems have been proposed in the literature based on the different characteristics of periodontal splints:

5.1. Goldman, Cohen, and Chacker Classification: This classification is based primarily on the duration of splint use. Temporary splints are subdivided into extracoronal types (e.g., wire ligation, orthodontic bands, removable acrylic or cast appliances, bonded materials) and intracoronal types (e.g., wire-and-acrylic, wire-and-amalgam, cast chrome-cobalt bars); provisional splints include all-acrylic and adapted metal band-and-acrylic splints; and permanent splints include etched bonded metal retainers and partial- or full-coverage fixed prostheses (Bhuvaneswari et al., 2019; Blessyphilip, Arunmozhi, Jacob, & Rajasekar, 2019).

5.2. Ross, Weisgold, and Wright Classification: This classification groups splints according to the intended period of stabilization into temporary stabilization (removable and fixed extracoronal, intracoronal, and etched metal resin-bonded splints), provisional stabilization (acrylic and metal-band-and-acrylic splints), and long-term stabilization (removable, fixed, and combined removable-fixed splints) (Bhuvaneswari et al., 2019; Gupta et al., 2023).

5.3. Grant, Stern, and Listgarten Classification: This classification groups splints according to their design into removable (external) splints, such as continuous clasp devices, swing-lock devices, and overdentures; fixed (internal) splints, such as full-coverage, three-quarter-coverage, and inlay restorations, posts in root canals, and horizontal pin splints; cast-metal resin-bonded fixed partial dentures (Maryland splints); combined removable-fixed designs; and other types, such as arch-bar and orthodontic wire-and-bracket splints (Bhuvaneswari et al., 2019; Blessyphilip et al., 2019; Grant, 1988).

5.4. Ramfjord Classification: Ramfjord's classification divides splints into temporary splints, which include fixed external types used for approximately 2-6 months (e.g., ligature wire, orthodontic bands) and removable types (e.g., removable partial dentures, night guards, removable acrylic splints); provisional splints, used for approximately 8-12 months for diagnostic purposes in borderline cases in which the outcome of treatment cannot be predicted; and permanent splints, which may be fixed, semirigid, or removable (Sood & Kaur, 2015).

5.5. Classification by Duration of Use and Site of Application

In addition to these author-based systems, periodontal splints can also be classified according to several basic criteria, including the duration of clinical use, the method of application to the tooth structure, the need for tooth preparation, and whether they can be removed by the patient.

The three duration-based categories shared by the classifications above can be defined as follows (Kathariya et al., 2016). Temporary splints are short-term appliances generally intended to remain in the oral cavity for less than six months (Blessyphilip et al., 2019; Kathariya et al., 2016). They are used during active periodontal therapy or following acute dental trauma to provide stabilization of the affected teeth and support tissue healing (Gürses & Öncü, 2019). Semi-permanent (provisional) splints are generally designed for use from several months to one or two years. In borderline cases, they serve as transitional appliances for diagnostic observation, allowing the long-term biological response of the periodontium to occlusal loading and treatment to be evaluated (El-Hassan, Peeran, & Ramalingam, 2021; Sharma, 2017). Permanent splints are long-term appliances designed to control persistent pathological tooth mobility and secondary occlusal trauma associated with reduced periodontal support, even after the completion of active periodontal therapy (Gürses & Öncü, 2019).

Based on the site of application and the need for tooth preparation, splints can also be classified into two main categories: extracoronal and intracoronal splints (Kathariya et al., 2016). Extracoronal splints are based on the direct bonding of the stabilization material to the external tooth surface without tooth preparation, whereas intracoronal splints are placed within grooves prepared on the lingual or occlusal surfaces of the teeth (Barzilay, 2000).

6. MATERIALS USED IN PERIODONTAL SPLINTING

In modern periodontal practice, clinicians have increasingly preferred materials with high biocompatibility, acceptable esthetic properties, and, most importantly, sufficient physiological flexibility to support periodontal healing (Kathariya et al., 2016; Rudo & Karbhari, 1999). With the introduction of adhesive resin composites, conventional wires began to be combined with composite materials. More recently, advances in dental material science have led to the widespread use of fiber-reinforced composite (FRC) systems and titanium trauma splints, which combine sufficient strength and flexibility with favorable esthetics and simple application and removal (Aslan & Acun Kaya, 2022; Gürses & Öncü, 2019; von Arx, Filippi, & Lussi, 2001).

6.1. Wire Ligatures

Wire ligatures, one of the traditional methods of fixation, have largely lost their role in routine clinical practice with the development of adhesive materials. However, they may still be used as an alternative fixation method when adhesive systems are unavailable or in emergency surgical situations. Due to their rigid structure and limited flexibility, these systems may restrict physiological tooth mobility and potentially interfere with periodontal ligament microcirculation and healing (Kahler et al., 2016; Mazzoleni et al., 2010). In addition, mechanical irritation of the periodontal soft tissues by rigid wires and increased plaque

accumulation, which may contribute to local inflammation, have considerably limited their use in contemporary periodontal therapy (Kahler et al., 2016).

6.2. Composite-Wire Splints

In this commonly used clinical technique, the orthodontic stainless steel wire is first passively adapted to the anatomical contour of the dental arch. After passive adaptation, the enamel surfaces of both the mobile teeth and the stable adjacent teeth used for anchorage are etched and bonded. The wire is then secured to the tooth surfaces with composite resin, creating a single splinted unit (Altan & Coşgun, 2016; Aslan & Acun Kaya, 2022; von Arx, Filippi, & Lussi, 2001). The main advantages of this design are the low cost and routine availability of the required materials (Altan & Coşgun, 2016; Aslan & Acun Kaya, 2022). However, because no chemical bond is formed between the metal wire and the composite resin, retention relies primarily on mechanical interlocking. Over time, repeated masticatory loading may lead to stress accumulation at the interface, resulting in restorative complications such as composite wear, debonding of the wire, or fracture (Pollack, 1999).

6.3. Composite Splints

Splints fabricated directly from composite resin are temporary or semi-permanent restorations commonly used to provide short-term mechanical support for tissue healing following acute dental trauma, periodontal surgery, or initial periodontal therapy (Kathariya et al., 2016). The application procedure involves preparing the interproximal and coronal enamel surfaces of the teeth to be stabilized according to adhesive protocols, followed by bonding the teeth together with composite resin to form a single unit. Although this technique is simple to apply and provides favorable esthetics, the inherent brittleness of composite resin may lead to fractures in the interdental areas under occlusal loading (Andreasen, 1981; Neaverth & Goerig, 1980). In addition, the bulk of composite resin in the interproximal areas may increase the risk of iatrogenic damage to intact enamel during splint removal and may promote plaque accumulation along the gingival margin, potentially leading to local gingival irritation (Filippi, Von Arx, & Lussi, 2002).

6.4. Bracket Splints

In this fixation technique, orthodontic brackets are bonded to the buccal surfaces of the affected and adjacent stable teeth with composite resin following standard adhesive procedures. An orthodontic archwire is then inserted into the bracket slots and secured with ligatures to complete the splinting system (Altan & Coşgun, 2016; Aslan & Acun Kaya, 2022). Because the occlusal relationship can be corrected orthodontically at a later stage, this technique is particularly useful for teeth that have been intruded or not correctly repositioned after trauma (Gupta et al., 2023). To provide adequate flexibility and permit the physiological

tooth mobility required for periodontal tissue healing, light flexible wires, such as 0.014-inch nickel-titanium (Ni-Ti), are recommended, and orthodontic forces should be avoided during healing (Gupta et al., 2023; Kahler et al., 2016).

6.5. Titanium Trauma Splint (TTS)

The titanium trauma splint (TTS), developed by von Arx et al. (von Arx, Filippi, & Buser, 2001), is a flexible titanium splint, 0.2 mm thick and 2.8 mm wide, with a rhomboid mesh design (Aslan & Acun Kaya, 2022; Gupta et al., 2023). It is bonded to the teeth with flowable composite resin, with one rhomboid opening per tooth filled with composite (Gupta et al., 2023; von Arx, Filippi, & Lussi, 2001). Its short application and removal times (von Arx, Filippi, & Lussi, 2001), preservation of physiological tooth mobility (Berthold, Thaler, & Petschelt, 2009; von Arx, Filippi, & Lussi, 2001), and good patient acceptance (Filippi et al., 2002) make it a suitable option in dental trauma, particularly for young patients (Altan & Coşgun, 2016; Aslan & Acun Kaya, 2022). However, its relatively high cost is a disadvantage (Aslan & Acun Kaya, 2022; Gupta et al., 2023).

7. FIBER-REINFORCED COMPOSITE (FRC) SPLINTS AND FIBER TYPES

FRCs consist of a polymer matrix, reinforcing fibers, and the interphase between them; the fibers are the load-bearing phase and hinder crack propagation, thereby improving the flexural strength, fracture toughness, and fatigue resistance of the composite. Various reinforcing fibers with different structural, mechanical, and esthetic properties have been described in the literature for use in FRC systems. The main fiber types used for this purpose include carbon fibers, aramid (Kevlar) fibers, glass fibers (e.g., E-glass and S-glass), and ultra-high-molecular-weight polyethylene (UHMWPE) fibers (Mangoush et al., 2017; Scribante, Vallittu, & Özcan, 2018).

7.1. Carbon Fibers

Carbon fibers can increase the flexural strength and stiffness of PMMA-based materials; however, their dark gray coloration may limit their use in esthetically demanding applications (Yerliyurt, Taşdelen, Eğri, & Eğri, 2023). Consequently, glass and polyethylene fibers are the fiber types most commonly used in dentistry (Mangoush et al., 2017).

7.2. Aramid (Kevlar) Fibers

Aramid (Kevlar) fibers are synthetic para-aramid (aromatic polyamide) fibers that are chemically resistant, thermally stable, and mechanically stable; however, their poor esthetics and difficulty in polishing have limited their intraoral use (Meenakshi Khandelwal, Punia, & Sharma, 2019).

7.3. Glass Fibers (E-Glass and S-Glass)

Glass fibers are inorganic, amorphous fibers based on a silica network and are classified by composition (e.g., E-glass and S-glass). They combine high tensile strength with low extensibility, are translucent, and can bond chemically to the resin matrix through silane coupling agents (Mangoush et al., 2017; Scribante et al., 2018). E-glass FRC reaches high flexural strength when positioned on the tension side (Lassila & Vallittu, 2004). However, under flexural loading, unidirectional E-glass-reinforced composites failed in a brittle manner, whereas UHMWPE braid- and leno-weave-reinforced composites deformed without rüptüre (Karbhari & Strassler, 2007).

7.4. Ultra-High-Molecular-Weight Polyethylene (UHMWPE) Fibers and Ribbond Technology

UHMWPE fibers are among the fiber types used in periodontal splinting due to their high strength and flexibility (Kathariya et al., 2016; Strassler et al., 2003). They are flexible, impact-resistant, low-density, and biocompatible, and their white, translucent appearance allows them to blend with the composite resin, providing favorable esthetics (Mangoush et al., 2017; Meenakshi Khandelwal et al., 2019; Spyrides, Prado, Araujo, Simão, & Bastian, 2015).

The favorable clinical performance of UHMWPE fibers is largely attributed to the biomechanical advantages provided by cold-gas plasma technology and the leno-weave architecture. Because polyethylene fibers are inherently hydrophobic, chemically inert, and have low surface energy, they adhere poorly to resin matrices. Cold-gas plasma treatment during manufacturing roughens the fiber surface and introduces oxygen-containing polar groups, increasing surface energy and making the surface hydrophilic, which improves resin impregnation and fiber-resin adhesion (Fernandes et al., 2026; Spyrides et al., 2015; Strassler et al., 2003). Nevertheless, adhesion to polyethylene remains less favorable than that achieved with silanized glass fibers (Mangoush et al., 2017). In addition, the patented leno-weave (lock-stitch) architecture maintains structural integrity and provides multidirectional reinforcement that helps prevent crack propagation (Strassler et al., 2003). The ribbon does not unravel after cutting and remains dimensionally stable, and the weave restricts fabric shearing during placement, facilitating handling and adaptation to tooth contours (Fernandes et al., 2026; Karbhari & Strassler, 2007; Strassler et al., 2003).

7.5. Comparative Overview of Splinting Materials

The main clinical advantages, limitations, and typical indications of the splinting materials described in Sections 6 and 7 are summarized in Table 1.

Splint type	Main advantages	Main limitations	Typical clinical use
Wire ligatures	Can be applied without adhesive systems	Rigid; may restrict physiological mobility and PDL healing Soft-tissue irritation and plaque accumulation	Emergency situations or when adhesive systems are unavailable
Composite-wire splints	Widely available materials Low cost	No chemical bond between wire and composite Wear, wire debonding, or fracture under repeated loading	Stabilization after dental trauma and of periodontally mobile teeth
Composite splints	Simple technique Favorable esthetics	Brittle; interdental fractures Risk of enamel damage on removal Plaque accumulation at the gingival margin	Short-term support after trauma, periodontal surgery, or initial therapy
Bracket splints	Facilitate subsequent orthodontic realignment Light flexible wires (e.g., 0.014-inch Ni-Ti) allow physiological mobility	Requires orthodontic brackets and archwire	Intruded or incompletely repositioned teeth after trauma
Titanium trauma splint (TTS)	Short placement and removal time Allows physiological tooth mobility Good patient acceptance	Relatively high cost	Dental trauma, particularly in pediatric patients
FRC splints (glass, UHMWPE)	Distribution of occlusal loads among the splinted teeth Sufficient strength and flexibility; favorable esthetics	Composite wear, loss of marginal adaptation, or debonding over time Plaque accumulation if embrasures are not properly designed Unidirectional glass fibers fail in a more brittle manner than woven polyethylene fibers	Short- and long-term stabilization of periodontally compromised mobile teeth

Table 1. Comparison of commonly used periodontal splinting materials.
Source: Compiled from (Altan & Coşgun, 2016; Aslan & Acun Kaya, 2022; Berthold et al., 2009; Filippi et al., 2002; Gupta et al., 2023; Gürses & Öncü, 2019; Kahler et al., 2016; Karbhari & Strassler, 2007; Kathariya et al., 2016; Kurgan et al., 2014; Liu et al., 2022; Mazzoleni et al., 2010; Neaverth & Goerig, 1980; Pollack, 1999; von Arx, Filippi, & Lussi, 2001; Zhang et al., 2023)
FRC: fiber-reinforced composite; Ni-Ti: nickel-titanium; PDL: periodontal ligament; UHMWPE: ultra-high-molecular-weight polyethylene.

8. CLINICAL PROTOCOL FOR DIRECT FRC SPLINTING OF MANDIBULAR ANTERIOR TEETH

A lingually bonded FRC splint on the mandibular anterior teeth can be completed in a single visit (Strassler et al., 2003). The following protocol summarizes the clinical steps described for a canine-to-canine UHMWPE ribbon splint (Strassler et al., 2003), supplemented by the maintenance protocols reported in long-term clinical studies (Sonnenschein et al., 2017; Zhang et al., 2023).

8.1. Pre-Splinting Assessment and Preparation

Indication and mobility assessment. The need for splinting should be based on a documented increase in tooth mobility, graded according to Miller's index (score 1: mobility greater than normal; score 2: horizontal mobility of up to 1 mm; score 3: more than 1 mm, combined with vertical depressibility) (Miller, 1950; Sood & Kaur, 2015). Progressive mobility, tooth migration, and pain on function are the main clinical reasons for stabilization (Strassler et al., 2003).

Completion of periodontal therapy. Initial periodontal therapy should be completed before splinting, and the patient's oral hygiene and motivation should be evaluated (Gürses & Öncü, 2019). In the protocol described by Strassler et al., scaling and root planing were completed before the splinting visit (Strassler et al., 2003).

Occlusal adjustment and abutment selection. Occlusal adjustment should be performed in most cases before splinting, and the stable teeth included in the splint should provide a greater root surface area than the mobile teeth (Bhuvaneswari et al., 2019). As a practical rule, at least two firm teeth should be included for every mobile tooth (Gupta et al., 2023).

8.2. Chairside Procedure

Step 1 - Isolation. The operative field is isolated with a rubber dam, which also protects exposed root surfaces (Strassler et al., 2003).

Step 2 - Cleaning. The facial and lingual surfaces are cleaned with a prophylaxis cup and nonfluoridated pumice, and the interproximal surfaces with finishing strips (Strassler et al., 2003).

Step 3 - Interproximal preparation. Where esthetics is a concern, a thin, round-ended chamfer diamond is used to reduce interproximal bulk, allowing the connectors to be placed without overcontouring and giving the illusion of non-splinted teeth (Strassler & Serio, 2007; Strassler et al., 2003).

Step 4 - Measuring and cutting the ribbon. The required length is measured on the lingual surfaces with dental floss from the midpoint of one canine to the midpoint of the contralateral canine. The plasma-treated ribbon is handled only

with clean cotton pliers to avoid surface contamination and is cut with the manufacturer's serrated scissors (Strassler et al., 2003).

Step 5 - Wetting the ribbon. The ribbon is wetted with unfilled resin, the excess is blotted off, and the ribbon is kept covered from light until placement (Strassler et al., 2003).

Step 6 - Etching. The enamel is etched with 35% phosphoric acid for 30 seconds, keeping the etchant away from exposed root surfaces, then rinsed and dried (Strassler et al., 2003; von Arx, Filippi, & Lussi, 2001). Wedges should be avoided because they may displace highly mobile teeth, which would then be splinted in an altered position; matrix strips can be placed distal to the terminal teeth instead (Strassler et al., 2003).

Step 7 - Blocking out the gingival embrasures. The gingival embrasures are passively blocked out with a medium- or heavy-body polyvinyl siloxane material so that the splint does not occlude the interdental spaces needed for oral hygiene (Sharma, 2017; Strassler et al., 2003).

Step 8 - Adhesive and interproximal composite. Adhesive is applied to the etched enamel, and a small amount of composite is placed in the facial interproximal areas and light-cured to seal the contacts, because these surfaces cannot be adequately cleaned once the teeth are splinted (Strassler et al., 2003).

Step 9 - Embedding the ribbon. Composite resin is placed on the lingual surfaces at the level of the middle third of the teeth, and the ribbon is embedded into it from canine to canine and adapted with cotton pliers and a burnisher. Excess material is removed, and each tooth is light-cured (Strassler et al., 2003).

Step 10 - Covering the ribbon. Any exposed fibers are covered with a high-strength flowable composite and light-cured (Strassler et al., 2003).

Step 11 - Finishing and polishing. The block-out material is removed, and the lingual surface is finished with egg-shaped finishing burs and polishing points. Interproximal areas are finished with reciprocating instruments rather than rotary burs, which can easily notch root surfaces (Strassler et al., 2003).

Step 12 - Occlusal check and hygiene instruction. Occlusion is checked and adjusted to eliminate premature contacts, and the patient is instructed in the use of interdental brushes appropriate for the splinted area (Strassler et al., 2003; Zhang et al., 2023).

8.3. Follow-Up and Maintenance

Splinted patients should be enrolled in supportive periodontal therapy. In long-term clinical studies, recall visits were scheduled at least once a year or two to three times a year and included plaque and bleeding scores, periodontal probing, oral hygiene reinforcement, and professional debridement

(Sonnenschein et al., 2017; Zhang et al., 2023). Periodontal and caries risk assessment during these visits is critical for splint longevity (Sharma, 2017).

The splint itself should be examined at every visit, since technical complications are common. Splint survival without fracture or debonding has been reported as 74.4% at three years (Sonnenschein et al., 2017) and 70.2% at five years (Zhang et al., 2023), and 75.3% of splints required repair during long-term maintenance (Graetz et al., 2019). An unnoticed debonding may compromise splint stability and plaque control; therefore, fractured or partially debonded segments should be repaired promptly (Strassler et al., 2003).

8.4. Splint Removal

Removal of bonded splints inevitably carries a risk of iatrogenic enamel damage (Kahler et al., 2016). The splint is sectioned interdentally, and the residual composite is removed carefully, preferably under magnification; tungsten carbide burs and polishing discs cause less enamel damage than debonding pliers, scalers, or diamond burs (Gupta et al., 2023; von Arx, Filippi, & Lussi, 2001). After removal of trauma splints, application of a fluoride solution to the treated surfaces has been recommended (von Arx, Filippi, & Lussi, 2001).

9. IMPORTANCE OF SPLINTING IN PERIODONTAL TREATMENT AND CURRENT EVIDENCE

Periodontal splinting is not an independent treatment modality that replaces periodontal therapy; rather, it is a complementary stabilization approach used in conjunction with active periodontal therapy and supportive periodontal care. Clinical studies have reported high survival rates for splinted teeth with reduced periodontal support receiving periodontal therapy and supportive periodontal care. However, the independent contribution of splinting to tooth survival remains uncertain (Dommisch et al., 2022; Graetz et al., 2019; Sonnenschein et al., 2017). However, differences in study designs, patient populations, and follow-up periods should be considered when interpreting the reported survival outcomes. Graetz et al. reported that, among patients with periodontitis followed for an average of 11 years, the risk of tooth loss was not significantly higher for splinted teeth than for non-splinted teeth (Graetz et al., 2019). Similarly, Sonnenschein et al. reported 100% tooth survival during the first three years for splinted mandibular anterior teeth followed for 3-15 years (Sonnenschein et al., 2017). Parthiban reported a survival rate of 99.6% (231 of 232 splinted teeth) over a mean follow-up of 8.5 ± 2.5 months during periodontal therapy, while Zhang et al. reported that none of the splinted teeth were extracted over a mean follow-up of 5.44 years (Parthiban, 2022; Zhang et al., 2023). These findings suggest that appropriately planned splinting combined with supportive periodontal care may be compatible with the retention of teeth with advanced mobility.

However, based on a low level of evidence, the systematic review by Dommisch et al. concluded that tooth splinting does not improve the survival of mobile teeth in patients with advanced periodontitis, with weighted mean tooth loss over 2 years of 8.4% for splinted and 10.1% for non-splinted teeth (Dommisch et al., 2022). Regarding periodontal tissue stability, the probing depth of splinted mandibular anterior teeth decreased and alveolar bone remained stable during supportive periodontal care (Zhang et al., 2023). In addition, Su and Cai reported further reductions in probing depth and attachment level values one month after splinting performed following periodontal treatment, and Tokajuk et al. reported a mean reduction in probing pocket depth of 0.58 mm, together with decreases in bleeding and gingival indices, 10 months after stabilization with a glass fibre splint (Su & Cai, 2018; Tokajuk, Pawińska, Stokowska, Wilczko, & Kedra, 2006).

In addition to clinical periodontal parameters, the effect of splinting on OHRQoL has been investigated. In a pilot randomized clinical trial of 28 re-evaluated patients, OHRQoL improved after non-surgical periodontal therapy in both groups, and the reduction in OHIP-G14 scores was considerably greater, although not statistically significant ($p = .080$), when mobile mandibular incisors were additionally splinted, suggesting a possible tendency toward further improvement (Sonnenschein et al., 2021).

10. CONCLUSION

Bone and attachment loss associated with periodontitis may reduce periodontal support, leading to pathological tooth mobility and related functional discomfort. Periodontal splinting is a complementary treatment approach that aims to control mobility by connecting mobile teeth to more stable adjacent teeth and distributing occlusal forces over a wider area.

With advances in dental material technology, fiber-reinforced systems, including those based on UHMWPE fibers, have become widely used for periodontal and restorative splinting because of their flexibility, mechanical properties, and favorable esthetic characteristics. When appropriately indicated and combined with effective periodontal therapy and supportive periodontal care, splinting may help improve functional comfort and facilitate the maintenance of mobile teeth. However, current evidence regarding its effect on long-term tooth survival and periodontal outcomes remains limited and heterogeneous.

Therefore, periodontal splinting should not be considered a substitute for periodontal therapy, but rather as an adjunctive treatment option that may be incorporated into comprehensive periodontal and restorative management according to individual patient needs.

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CHAPTER 15

Biomimetic Approaches in Indirect Posterior Restorations: New-Generation Restorative Materials and Surface Adhesion Protocols

Sedef Kurt Çıralık[1]

[1] Arş. Gör, Recep Tayyip Erdoğan Üniversitesi Diş Hekimliği Fakültesi, ORCID iD:0009-0004-7454-5369

1.Biomimetic Dentistry Philosophy

For many years, the conventional approach to prosthodontics was based on macro-mechanical preparation principles centered on retention and resistance form. Although this approach may ensure the retention of restorations in the oral cavity, it may also result in excessive loss of healthy dental tissue and disruption of the tooth's biomechanical balance (1). The biomimetic dentistry philosophy that has emerged in modern prosthodontics is based on the principle of "imitating nature." Its primary goal is to preserve pulp vitality and restore lost hard tissues in a tissue-friendly manner using materials that most closely approximate the original anatomical and mechanical properties of the tooth, including elastic modulus, strength, and light transmission (1,2). Three fundamental stages of a biomimetic approach in the posterior region can be identified (1,3):

Maximum preservation of hard tissues: Preparation margins should be determined according to the anatomical boundaries of the defect rather than according to predetermined preparation angles.

Optimization of stress distribution: The natural biomechanical balance between rigid enamel and more compliant dentin should be approximated.

Preservation of hybrid-layer integrity: Advanced surface pretreatment protocols should be used to support the long-term performance of the adhesive interface.

2. Types of Indirect Restorations in the Posterior Region and Clinical Decision-Making

In posterior teeth with extensive loss of tooth structure, direct composite restorations may be susceptible to failure because of polymerization shrinkage, marginal leakage, and inadequate occlusal contacts (2). Indirect and semi-direct techniques may overcome some of these limitations.

2.1. Indirect Restoration Geometries

The selection of indirect restoration geometries for managing hard-tissue loss in posterior teeth depends on cavity depth, the amount of remaining tooth structure, and the degree of cuspal support. Inlays are generally indicated in limited cases without cuspal loss and with sufficiently supported cavity walls; however, their indications have become more restricted within a biomimetic treatment philosophy (2). In contrast, when at least one cusp is weakened or fractured, onlay restorations incorporating cuspal coverage may be preferred to facilitate a more favorable distribution of occlusal loads (3). When the loss of tooth structure is more extensive and the entire occlusal table needs to be covered while protecting the remaining cusps, overlay restorations may be considered as a minimally invasive design (3). Finally, in extensively damaged endodontically treated posterior teeth, endocrowns have emerged as monolithic restorative

options that use the depth and retention of the pulp chamber and may eliminate the need for conventional post-and-core procedures (4).

2.2. Deep Margin Elevation (DME)

In the presence of subgingival margins, digital or conventional impression procedures, isolation, and cementation become considerably more challenging. The DME technique involves relocating a subgingival cervical margin to a supragingival position using a matrix band and composite resin (5,6). DME may facilitate isolation and restorative procedures in cases with deep cervical margins; however, its long-term periodontal and adhesive benefits should be interpreted in light of the available evidence.

3. New-Generation Restorative Materials and Biomimetic Comparison

Material selection for indirect posterior restorations should consider resistance to masticatory forces, wear of the opposing dentition, and an elastic modulus compatible with tooth tissues (7).

3.1. Glass Ceramics

• Lithium disilicate ceramics: With high flexural strength (approximately 350–500 MPa) and favorable esthetic and adhesive characteristics, lithium disilicate is widely used for indirect posterior restorations (3,7).

• Zirconia-reinforced lithium silicates (ZLS): These glass-ceramic materials contain zirconium oxide and are characterized by a fine crystalline microstructure, offering favorable processing characteristics and surface quality (7).

3.2. Resin-Matrix Ceramics and Hybrid Blocks

• Polymer-infiltrated ceramic networks (PICNs): These materials are produced by infiltrating a porous ceramic network with a polymer matrix (e.g., VITA Enamic) (9).

• Resin nanoceramics (RNCs): These are CAD/CAM materials containing a high proportion of nano-sized fillers (9).

Biomimetic advantage: The elastic modulus of some resin-matrix ceramic and hybrid materials (approximately 15–20 GPa) is relatively close to that of human dentin (approximately 18 GPa). This similarity may contribute to stress attenuation and may influence stress transfer to the underlying tooth tissues (7,9).

3.3. Translucent Monolithic Zirconia

New-generation 4Y-PSZ and 5Y-PSZ zirconia materials, with increased cubic-phase content, combine high strength with improved optical properties (8,10). They may be considered in patients with parafunctional habits and in cases

requiring conservative reduction, such as selected overlay or endocrown restorations (4,8).

4. Surface Pretreatment and Adhesion Protocols

The success of adhesive dentistry depends substantially on appropriate conditioning and treatment of both the tooth–adhesive interface and the internal surface of the restoration (13).

4.1. Tooth-Surface Protocols and Immediate Dentin Sealing (IDS)

Immediate dentin sealing (IDS) is an important component of biomimetic adhesive restorative protocols (1,11,12).

Clinical advantages of IDS:

• Immediate hybridization of freshly cut dentinal tubules may reduce postoperative sensitivity (11).

• The adhesive interface can mature before exposure to provisional-restoration materials and oral contamination (1,12).

• Immediate sealing has been associated with higher bond-strength values than delayed dentin sealing in many in-vitro investigations (12).

4.2. Restoration Surface Pretreatment Protocols

The recommended surface-conditioning protocol varies according to the chemical composition and micromechanical characteristics of the restorative material (13,14).

A. Glass ceramics (lithium disilicate and ZLS)

1. Hydrofluoric acid (HF) etching: HF is used to etch the internal surface of glass ceramics. The exact concentration and etching time should follow the manufacturer's instructions because they vary according to the ceramic composition (7,14).

2. Cleaning after etching: After HF etching, the surface should be thoroughly rinsed and cleaned to remove reaction products and residues before silanization.

3. Silanization: A suitable silane coupling agent is applied according to the manufacturer's instructions. Silane promotes chemical coupling between the silica-containing ceramic surface and the organic resin cement (13,14).

B. Resin-matrix ceramics and hybrid blocks

1. Air abrasion: Controlled air abrasion with alumina particles may be used according to the specific CAD/CAM material and manufacturer's recommendations. Excessive air-abrasion pressure may damage resin-based CAD/CAM blocks (9,16).

2. Cleaning and silanization/adhesive application: Following surface cleaning, a silane-containing agent or an adhesive system containing an appropriate functional monomer such as 10-MDP may be selected according to the restorative material and manufacturer's instructions (13,16).

C. Zirconia ceramics (APC concept)

Because zirconia does not contain a glass phase, hydrofluoric acid etching is not an effective surface-conditioning method. An air-abrasion–primer–composite (APC) concept is commonly recommended (15).

Air-abrasion: Controlled air abrasion with alumina or silica-coated alumina particles may be used at a pressure appropriate for the zirconia system (15).

Primer: A zirconia primer or universal adhesive containing 10-MDP may be applied. Functional phosphate monomers can chemically interact with zirconia oxide surfaces (13,15).

Composite resin: A compatible resin cement, commonly a dual-cure resin cement when indicated, is used for definitive cementation (15,17).

5. Adhesive Cementation Protocol and Clinical Tips

Adhesive cementation is a critical final stage that facilitates load transfer between the restoration and the tooth (17).

5.1. Cement selection:

• Dual-cure resin cements: In indirect restorations where light transmission is limited, dual-cure resin cements may be preferred because they combine light- and chemical-curing mechanisms (17).

• Preheated composite resin: For thin or highly translucent indirect restorations such as onlays and overlays, preheating composite resin can reduce viscosity and facilitate seating. The literature reports a range of preheating temperatures, with approximately 54–68°C among the most frequently reported values; protocols should be standardized according to the material and clinical system used (18).

6. Clinical Complications and Management of Failures

Although indirect posterior restorations generally demonstrate favorable clinical performance and survival, complications may arise from material selection, preparation geometry, occlusal load distribution, or technical deficiencies during adhesive cementation (19). Accurate diagnosis and classification of complications, followed by minimally invasive management consistent with biomimetic principles, are important for preserving tooth structure and extending restoration longevity (19,20).

6.1. Adhesive Failures and Loss of Bond (Debonding)

Debonding is a mechanical/biological complication characterized by loss of retention of the restoration and/or marginal defects. It may be associated with contamination by saliva or blood during cementation, inappropriate conditioning of the restoration's internal surface, inadequate silanization, or insufficient control of polymerization-related stresses (20).

When IDS is not performed immediately after dentin preparation, the exposed dentin may become contaminated during the provisional phase, potentially compromising the adhesive interface (11,12). If debonding occurs while the restoration remains intact and marginal adaptation is acceptable, the restoration and tooth surfaces may be reconditioned and the adhesive cementation protocol repeated when clinically appropriate (20).

6.2. Ceramic and Material Fractures (Chipping vs. Catastrophic Fracture)

Mechanical fractures in indirect posterior restorations can broadly be considered as localized chipping or more extensive structural fractures (21).

1. Chipping: Localized fracture may occur when ceramic or hybrid materials are subjected to stresses that exceed their local tolerance, when occlusal adjustment is inadequate, or when restoration thickness is insufficient. If overall restoration integrity and marginal adaptation are maintained, intraoral repair may be considered rather than complete replacement (21,22).

2. Catastrophic structural fractures: These involve extensive fracture of the restoration and/or underlying tooth structure. Risk may increase in patients with parafunctional habits, inappropriate material selection, inadequate cuspal coverage, or pre-existing deep cracks (21). In extensive fractures, the restoration should be removed and the remaining tooth structure reassessed biomechanically; an overlay or endocrown design may be considered when indicated (4,21).

6.3. Biological Complications: Secondary Caries and Pulpal Reactions

Secondary caries may be associated with marginal leakage, inadequate finishing and polishing, inappropriate margin design, and plaque accumulation around subgingival margins (23). In the presence of subgingival margins, inadequate execution of DME may also adversely affect periodontal conditions (5,6).

Failure to seal exposed dentin before cementation or failure to identify premature occlusal contacts after cementation may contribute to postoperative sensitivity and pulpal irritation (1,23). When secondary caries develops, localized repair or correction of the cervical margin may be considered where appropriate. If pulpal involvement occurs, endodontic treatment may be performed through an appropriate access cavity without necessarily removing an otherwise serviceable

restoration; the access cavity should subsequently be sealed with an appropriate restorative material (23).

6.4. Intraoral Ceramic Repair Protocol

For glass-ceramic, zirconia, or hybrid restorations exhibiting chipping while maintaining acceptable marginal integrity, an intraoral repair approach may help preserve tooth structure and avoid complete replacement (22,24).

1. Isolation and surface preparation: Isolate the area with a rubber dam. Roughen the fractured restoration surface and exposed tooth structure using an appropriate abrasive system or fine-grit diamond instruments.

2. Chemical surface activation:

• Glass-ceramic surfaces: Etch with hydrofluoric acid according to the manufacturer's recommended concentration and duration, then rinse and dry.

• Zirconia surfaces: After air abrasion, apply a zirconia primer or adhesive containing 10-MDP.

• Exposed tooth structure: Etch enamel and dentin with phosphoric acid according to the selected adhesive system's instructions.

3. Silanization and adhesive application: Apply a suitable silane agent to glass-ceramic surfaces when indicated, followed by the appropriate adhesive system and polymerization.

4. Restoration and polishing: Restore the fractured area incrementally with a suitable nano-hybrid or microfilled composite resin and finish/polish the repaired area (22,24).

Conclusion

In indirect posterior restorations, the biomimetic approach is not merely an esthetic preference; rather, it aims to preserve the tooth as closely as possible to its natural biomechanical and functional state. To achieve successful biomimetic restorative treatment:

1. Unnecessary loss of tooth structure should be avoided, and decisions regarding cuspal preservation and coverage should be based on biomechanical considerations (1,3).

2. Dentin should be sealed immediately after preparation when IDS is indicated (11,12).

3. Material selection should consider not only esthetics and fracture resistance but also compatibility of elastic modulus with tooth tissues (7,9).

4. Material-specific surface-conditioning protocols (e.g., HF/silane for glass ceramics or air abrasion/MDP for zirconia) should be followed according to current evidence and manufacturer recommendations (13–15).

In the future, digital scanning and artificial-intelligence-assisted CAD/CAM systems may facilitate the production of gradient or functionally graded restorative materials designed to reproduce the natural stress distribution of teeth, potentially advancing biomimetic dentistry.

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CHAPTER 17

Ionizing Radiation and Articular Cartilage: From Radiation Exposure to Cartilage Injury and Osteoarthritis

Ekrem Çiçek[1]

[1] Professor, Burdur Mehmet Akif Esoy University, Faculty of Art and Science, Department of Physics, Burdur, Türkiye, ORCID: 0000-0001-6724-9423

1. Introduction

1.1. Articular Cartilage Architecture

Hyaline articular cartilage consists of Type II collagen, large aggregating proteoglycans, water, and chondrocytes. Articular cartilage has four zones: superficial (tangential), transitional, deep radial, and calcified. It is entirely dependent on diffusion for metabolic exchange because it is avascular and lacks a direct vascular supply. This property fundamentally shapes its radiation response. (Sophia et al., 2009; Cicek et al., 2010; Cicek & Arikanoglu 2014; Loeser et al., 2016)

1.2. Defining Ionizing Radiation

IR includes electromagnetic radiation (X-rays, gamma rays) and particulate radiation (alpha/beta particles, protons, neutrons, carbon ions), all of which have sufficient energy (>10 eV) to ionize biological molecules. Linear Energy Transfer (LET), Relative Biological Effectiveness (RBE), and dose rate are among the fundamental physical parameters that govern tissue damage. Each determines the qualitative nature of biological damage (Azzam et al. 2012; Hall & Giaccia 2019; ICRP Pub. 103 2007)**.**

1.3. Clinical Relevance

Articular cartilage is exposed to IR in three principal circumstances: (i) diagnostic imaging (radiography, CT arthrography, and nuclear medicine); (ii) therapeutic radiotherapy for periarticular or synovial tumors such as synovial sarcoma and pigmented villonodular synovitis (PVNS); and (iii) incidental joint irradiation during treatment of adjacent malignancies (Saintigny et al., 2015; Naing et al., 2015; Cash & Dean 2019).

1.4. The Radioresistance Paradox

The low mitotic index of chondrocytes (<0.01% in adult tissue) historically supported the designation of cartilage as 'radioresistant'. This may reduce susceptibility to radiation-induced mitotic cell death. Nevertheless, this same characteristic eliminates the tissue's intrinsic regenerative capacity. Radiation-induced chondrocyte loss and ECM damage are effectively permanent and progressive joint destruction (Mankin 1982; Buckwalter & Mankin 1997; Archer & Francis-West 2003).

The unique radiobiology of articular cartilage is determined by the dichotomy between low acute cytotoxicity and irreversible chronic degeneration. Whereas some radiation effects may occur early, cartilage degeneration may develop over a longer period. These mechanisms cannot be detected using traditional methods

of early toxicity monitoring. This situation requires a fundamentally different paradigm for dosimetric constraints and long-term follow-up in radiotherapy around joints (Bentzen 2006; Saintigny et al. 2015; Dörr et al. 2017).

2. Articular Cartilage Structure and Radiation Physics

Table 1 summarizes the key physical and biological parameters of each IR modality relevant to articular cartilage injury.

Modality	**LET (keV/μm)**	**RBE**	**Clinical Dose Range**
Diagnostic X-rays (20–150 keV)	~0.3	1.0	0.01–10 mGy
Therapeutic photons (MV)	~0.2–0.3	1.0	45–70 Gy
Gamma rays (Co-60)	~0.2	1.0	Up to 60 Gy
Proton beams	0.5–10 (Bragg peak)	1.1	50–70 Gy(RBE)
Carbon ions (C-12)	~28 (Bragg peak)	2–3	48–60 Gy(RBE)
Neutrons	20–100	3–20	Occupational
Beta particles (radiosynovectomy)	~0.2	~1.0	Up to 100 Gy synovium
Alpha particles (Ra-223)	~100	~20	Targeted alpha-particle therapy
Electron beam	~0.2	~1.0	10–20 Gy

2.1. X-rays

Low-energy diagnostic X-rays (20–150 keV) deliver a minimal dose to cartilage per examination (0.01–10 mGy). Megavolt-range photon beams used for treatment (4–18 MV) deliver doses typically encountered in radiation therapy (45–70 Gy). Low LET (~0.2–0.3 keV/μm) damage is not efficiently repaired in senescent or slow-cycling chondrocytes (Cash & Dean, 2019; Chen et al., 2019).

2.2. Proton Beams

Proton beams deliver a high dose at depth with minimal exit dose. At the Bragg peak, LET rises substantially (5–10 keV/μm), increasing RBE to approximately 1.1. If the Bragg peak falls within articular cartilage biological damage per physical dose unit is amplified by ~10% compared to photons. This is a clinically relevant margin given the tissue's limited capacity for repair (McNamara et al., 2015).

2.3. Carbon Ions and Heavy Particles

Carbon ion therapy achieves exceptional dose conformality, LET up to 28 keV/μm, and RBE of 2–3. Positioning the carbon Bragg peak within joint structures risks disproportionate and potentially irreparable cellular injury for articular cartilage (Karger & Peschke 2017).

2.4. Nötronlar ve Alfa Parçacıkları

Neutrons (LET 20–100 keV/μm; RBE 3–20) are observed in occupational/accidental exposure and certain historical RT situations. Alpha particles (LET ~100 keV/μm; RBE ~20) are emitted by radionuclides such as Ra-223 (ICRP Pub. 103 2007).

2.5. Beta Particles and Electron Beams

Beta-emitting radionuclides (Y-90, Re-188, Er-169) are used in radiosynovectomy. Their low LET (~0.2 keV/μm) is biologically analogous to photons. Electron beam therapy potentially delivers a significant dose to the superficial cartilage zone (Kampen & Brenner, 2006).

3. Cellular Responses to Ionizing Radiation

3.1. Radiation-induced DNA Damage

IR generates DSBs and single-strand breaks (SSBs). In vitro studies demonstrate that human articular chondrocytes exhibit a persistent repair deficit ≥24 hours post-irradiation at doses as low as 2 Gy. This repair deficit is further exacerbated by the low metabolic activity characteristic of adult articular cartilage (Hong et al., 2010; Alessio et al., 2016).

3.2. Cell Cycle Arrest and Checkpoint Activation

Doses ≥4 Gy activate ATM → p53 → p21 signaling in chondrocytes, inducing G1/S and G2/M checkpoint arrest. Chondrocyte arrest frequently becomes permanent, shifting cells toward senescence rather than repair and proliferative recovery (Hong et al., 2010).

3.3. Apoptosis Versus Senescence: A Dose-Dependent Threshold

At doses below 10 Gy, Chondrocyte senescence prevails over apoptosis. Senescent chondrocytes adopt the senescence-associated secretory phenotype (SASP), creating a self-amplifying catabolic microenvironment and promoting systemic aggrecan degradation. SASP-positive senescent cells are thought to contribute to the chronic and progressive progression of radiation-induced cartilage injury (Hong et al., 2010; Battistelli et al., 2014; Stelcer et al., 2018).

3.4. Mitochondrial Dysfunction and Sustained ROS Generation

Radiation-induced mitochondrial membrane permeabilization generates sustained production of reactive oxygen species (ROS). This chronic oxidative burden further suppresses collagen synthesis, establishing a feed-forward injury cycle (Chen et al., 2008).

3.5. Impaired Chondrogenic Differentiation

Radiation doses of 2 Gy or higher, transcriptional shift promotes chondrocyte hypertrophy and phenotypic dedifferentiation, potentially driving endochondral ossification of articular cartilage (Takahashi et al., 2003).

4. Extracellular Matrix and Joint-Tissue Changes

4.1. Collagen Network Disruption

IR directly cleaves peptide bonds and collagen cross-links by generating hydroxyl radicals. Then, it disrupts fibril architecture and reduces tensile strength. It was demonstrated that progressive loss of birefringence in polarized light microscopy within weeks of irradiation in animal studies (murine and rabbit models, 20–40 Gy) (Willey et al., 2013; Cicek, 2017).

4.2. Proteoglycan and GAG Depletion

Upregulation of ADAMTS-4 and ADAMTS-5 (aggrecanases) after irradiation leads to aggrecan degradation. It was demonstrated that dose-dependent GAG loss began at approximately 20–30 Gy, with near-complete depletion at 50 Gy in irradiated rabbit cartilage. The tissue's compressive load-bearing function was impaired by this loss of GAGs (Cicek, 2016; Willey et al., 2013).

4.3. MMP Upregulation and Matrix Catabolism

MMP-1, MMP-3, and MMP-13 are transcriptionally activated, degrading Type II collagen and aggrecan simultaneously. This combined catabolic activity involves aggrecanases, which target proteoglycans, and collagenases, which target collagen fibrils (Willey et al., 2013; Hong et al., 2014).

4.4. Water Content Dysregulation

GAG depletion reduces the fixed charge density of the ECM, diminishing osmotic swelling pressure and water retention. The biomechanical changes resulting from this condition include decreased cartilage stiffness, increased tissue permeability, and altered load distribution (Han et al., 2011).

4.5. Subchondral Bone Involvement and Secondary Cartilage Failure

Radiation-induced injury to subchondral bone may contribute to secondary cartilage degeneration by altering the mechanical support provided to the overlying cartilage. Changes in subchondral bone structure and stiffness could modify stress distribution at the osteochondral interface. However, the contribution of this pathway to radiation-associated cartilage damage may vary with the anatomical site, dose distribution, and extent of bone injury (Hutchinson et al., 2014).

4.6. Persistent Anabolic Suppression

Synthesis of Type II collagen, lubricin, and aggrecan is chronically suppressed after irradiation. Radiation-damaged cartilage exhibits a near-complete anabolic deficit (Takahashi et al, 2003).

5. Effects of Diagnostic Radiation

5.1. Plain Radiography and Fluoroscopy

Effective doses per joint radiograph range from 0.001–0.1 mGy. This amount is below the experimentally established threshold for chondrocyte damage (>1 Gy in vitro). There is no consistent evidence that isolated diagnostic exposures cause direct injury to articular cartilage in adults (Saintigny et al, 2015).

5.2. CT Arthrography

Joint cartilage was exposed an estimated 5–20 mGy per CT arthrogram. It was not reproducibly demonstrated that chondrocyte apoptosis or ECM changes at these levels. Patients with inflammatory joint diseases who receive repeated fluoroscopy-guided joint injections over several years may build up radiation doses that come close to levels considered biologically significant.

5.3. Nuclear Medicine

Tc-99m MDP bone scans deliver an effective dose of approximately 4-6 mSv for the entire body. Since the agent's preferential uptake in subchondral bone, exposure to articular cartilage is minimal. FDG-PET cartilage uptake is physiologically low. No clinically meaningful cartilage toxicity has been attributable to standard nuclear medicine procedures (Nakamura et al., 2007; Kuzu & Cicek, 2019).

5.4. Pediatric Growth Cartilage — A Distinct and Critical Vulnerability

Growth cartilage is markedly more radiosensitive than adult articular cartilage due to its high mitotic rate and active endochondral ossification. Doses as low as 6–10 Gy can cause physeal arrest, angular deformity, and limb length discrepancy in children (Hutchinson et al., 2014). This is a distinct entity from adult articular cartilage injury and requires strict dose minimization and long-term orthopedic surveillance in all pediatric radiotherapy patients.

The linear no-threshold (LNT) model, while the regulatory standard for radiation protection, should not be conflated with biological evidence of actual cartilage injury at diagnostic doses. Current data strongly support the radiological safety of standard diagnostic imaging for adult articular cartilage. Longitudinal studies quantifying cumulative cartilage exposure in chronically imaged patient populations — a pressing gap in the literature — are needed to establish evidence-based upper limits for repeat diagnostic exposure.

6. Therapeutic Radiation and Clinical Joint Outcomes

6.1. Direct Joint Irradiation

Synovial sarcoma, pigmented villonodular synovitis (PVNS), tenosynovial giant cell tumor (TGCT), and giant cell tumors of the tendon sheath are among the conditions in which the radiation field may directly involve an articular joint. Histopathological examination of surgical specimens obtained after radiotherapy has shown reductions in chondrocyte density and depletion of glycosaminoglycans (GAGs) in patients exposed to cumulative radiation doses of 50–60 Gy. The extent of cartilage damage may vary according to the dose, fractionation schedule, and volume of cartilage exposed (Saintigny et al, 2015; Cicek, 2016).

6.2. Incidental Joint Irradiation During Cancer Radiotherapy

Joints may also receive radiation as an unintended consequence of treatment for tumors located in nearby anatomical regions. Pelvic radiotherapy, including treatment for prostate, cervical, and rectal cancers, can expose the hip and sacroiliac joints. Thoracic irradiation may involve the sternoclavicular and glenohumeral joints, while treatment of head-and-neck tumors can expose the temporomandibular joint (TMJ). Trismus and changes in the articular cartilage of the TMJ have been reported following relatively high radiation doses to this region (Kılıc et al., 2019). Spinal radiotherapy may similarly expose the facet joints and the cartilage associated with the intervertebral disc.

6.3. Radiation-induced Osteoarthritis (RIAO)

Radiation-induced osteoarthritis (RIAO) is generally characterized by a latency period of approximately 2–15 years. The clinical findings may include progressive narrowing of the joint space, subchondral sclerosis, and osteonecrosis. Histologically, RIAO may resemble end-stage primary osteoarthritis; however, it can occur in relatively younger patients without the traditional risk factors associated with primary OA. Incidence in pelvic RT recipients: 3–10%; substantially higher in direct joint irradiation scenarios. RIAO is systematically underdiagnosed due to limited long-term oncologic follow-up and the absence of imaging surveillance protocols (Saintigny et al, 2015; Hamdi et al., 2016).

6.4. Dose Thresholds for Cartilage Toxicity

The available data suggest that the TD5/5 (5% complication rate at 5 years) for cartilage or joint injury is approximately 50–60 Gy with conventional fractionation, while the TD50/5 may be around 65–70 Gy. However, these estimates should be interpreted with caution because they are based largely on heterogeneous retrospective studies and have not been adequately validated in randomized comparative studies. An EQD2 of ≤45–50 Gy ($\alpha/\beta = 3$ Gy) has been

proposed as a conservative planning constraint for articular cartilage, although the supporting evidence remains limited (Cash & Dean 2019).

6.5. Fractionation Schemes and The Potential Risks of SBRT and SRS

SBRT involves delivering relatively high doses per fraction, typically 6–24 Gy. Because cartilage is considered a late-responding tissue with a low α/β ratio, high-dose fractions may increase the risk of late radiation injury. For example, 45 Gy delivered in three fractions (15 Gy per fraction) corresponds to an EQD2 of approximately 315 Gy when an α/β ratio of 3 Gy is assumed. If a joint is located within the high-dose region, such a regimen could therefore present a substantial risk of cartilage damage. Cases of radiation-induced osteoarthritis following SBRT have been reported, with some patients developing changes within approximately 12–18 months (Saintigny et al, 2015).

6.6. Proton and Carbon Ion Therapy: Potential for Cartilage Sparing

Dosimetric studies have reported lower mean doses to joints with proton therapy than with photon-based IMRT in selected periarticular cases. Carbon ion therapy can provide dose distributions. But the increased relative biological effectiveness (RBE) near the Bragg peak introduces additional uncertainty when the treatment field involves articular cartilage. For patients with tumors located close to cartilage, proton therapy may therefore be considered when reducing radiation exposure to normal tissues is an important treatment objective (de Kruijff, 2020; Bogaerts et al., 2022; Cengel et al., 2024).

7. Radiation Modality and Dose–Response Considerations

The effects of ionizing radiation on articular cartilage depend on the radiation modality, dose, fractionation, and volume of cartilage exposed. Although most available evidence concerns external-beam radiotherapy, other exposure scenarios may also affect joint tissues.

7.1. Radiosynovectomy

Intra-articular radionuclides such as Y-90, Re-188, and Er-169 are used to treat inflammatory synovitis, particularly in rheumatoid arthritis and hemophilia-associated arthropathy. Experimental studies suggest that high radiation doses delivered to the synovium may also affect adjacent articular cartilage. However, long-term clinical data on cartilage outcomes after radiosynovectomy remain limited (Howson et al., 1988; Mäkelä et al., 2003; Rampersad et al., 2013).

7.2. Total Body Irradiation (TBI)

TBI used in conditioning regimens exposes the entire body to radiation and may contribute to late skeletal complications. In pediatric cancer survivors, joint and subchondral bone complications have been reported, although the specific

contribution of radiation to articular cartilage injury remains difficult to determine (Hutchinson et al., 2014; Alessio et al., 2017).

8. Potential Protective Strategies

Several approaches have been investigated to reduce radiation-induced cartilage injury, but most remain at the experimental stage.

8.1. Antioxidant and Anti-Inflammatory Approaches

Agents such as amifostine, vitamin E, and N-acetylcysteine have shown protective effects against radiation-induced oxidative stress and extracellular matrix degradation in experimental models. However, their efficacy for preventing cartilage injury has not been established in clinical studies.

8.2. Hyaluronic Acid and Platelet-Rich Plasma (PRP)

These approaches may reduce inflammation and support cartilage homeostasis after radiation exposure. Experimental studies, including animal models, have reported potentially beneficial effects, but clinical evidence remains limited (Kılıc et al., 2019).

8.3. Senolytic Strategies

Dasatinib and quercetin have shown beneficial effects in experimental models of chondrocyte senescence and osteoarthritis. Because radiation can promote chondrocyte senescence, senolytic therapy represents a potential strategy for reducing radiation-associated cartilage damage. However, this approach has not yet been clinically evaluated for radiation-induced cartilage injury (Zhao et al., 2025; Maurer et al., 2025).

8.4. Treatment Planning and Dose Reduction

The most immediately applicable strategy is to minimize unnecessary radiation exposure to joints through treatment planning and dose–volume optimization. Contouring articular cartilage as an organ at risk may help identify patients receiving substantial cartilage doses and facilitate future development of dose constraints.

9. Conclusion

9.1 Mechanistic Synthesis

Early alterations induced by ionizing radiation may include direct physicochemical damage to the extracellular matrix (ECM). These effects can be followed by chondrocyte senescence and alterations in the balance between anabolic and catabolic processes. Synovial changes may contribute to progressive joint degeneration in the longer term. making the long-term consequences of cartilage irradiation difficult to detect during routine follow-up. Clinical signs may occur years after radiotherapy.

9.2 Clinical Considerations Based on The Available Evidence

The current literature indicates several areas that may deserve attention in radiotherapy planning. Articular cartilage can be considered an organ at risk when the treatment field is close to it. Where clinically feasible, recording cartilage dose–volume parameters may help improve our understanding of dose-related toxicity. Proton therapy may also be considered for patients with tumors close to cartilage when reduction of radiation exposure to normal tissues is an important treatment objective. Longer-term orthopedic assessment could be useful in patients who receive substantial radiation doses to joint-containing regions.

9.3. Potential Risk Stratification

It could consider the radiation dose received by the cartilage, the patient's age, and whether the cartilage is directly included. Patients receiving relatively low joint doses would generally be expected to have a lower risk of clinically significant radiation-related joint injury. Higher doses, particularly those above 40 Gy, direct irradiation of the joint, may warrant closer long-term follow-up. Because of their longer expected survival and the potential for late radiation effects, pediatric patients may also require particular attention.

Cartilage has received less attention in studies of the late effects of radiation therapy. Cartilage has historically been considered relatively resistant to radiation. In literature, experimental and clinical findings show that radiation exposure can affect chondrocyte survival, extracellular matrix homeostasis, and joint tissues. A better characterization of radiation effects will become increasingly important as the number of long-term cancer survivors increases. Future radiotherapy studies should include a sufficiently long-term follow-up period to detect cartilage and joint damage.

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CHAPTER 18

The Effects of Electromagnetic Fields on Bone Mineral Density

Ekrem Çiçek[1]

[1] Professor, Burdur Mehmet Akif Esoy University, Faculty of Art and Science, Department of Physics, Burdur, Türkiye, ORCID: 0000-0001-6724-9423

1. Introduction

Osteoporosis is a systemic skeletal disorder characterized by diminished bone mass and deterioration of bone microarchitecture. It is resulting in heightened fracture susceptibility particularly in postmenopausal women and the elderly (NIH Consensus Development Panel, 2001). Pharmacological management while efficient, carries significant side impact profiles, compliance challenges, and high long-term costs (Fatoye et al., 2019; Blume & Curtis, 2011). The non-invasive therapies have led to growing interest in the biological effects of electromagnetic fields (EMF) on skeletal tissue (Wang et al., 2016).

The study of the effects of electromagnetic fields on bone dates back to the 1950s. During that period, it was demonstrated that mechanically loaded bone generates piezoelectric potentials and that endogenous electrical currents play a role in regulating bone metabolism (Fukada & Yasuda, 1957; Tabrah et al., 1998; Rubin et al., 1989). This observation led to the systematic study of exogenous EMF as a method to mimic or enhance the bone's intrinsic bioelectric environment. Following the approval of pulsed EMF for the treatment of non-healing fractures in 1979, the use of EMF therapy for the prevention and treatment of osteoporosis increased Tabrah et al., 1990).

There are various treatment methods using EMF (electromagnetic fields). These methods include: pulsed electromagnetic fields (PEMF), typically with frequencies between 5 and 300 Hz; extremely low-frequency EMF (ELF-EMF), defined as fields below 60 Hz; radiofrequency EMF (RF-EMF), which includes GSM mobile phone frequencies at 900 MHz and 1800 MHz; static magnetic fields (SMF); and pulsed electric field stimulation. Each method differs in physical parameters such as frequency, intensity, waveform, and exposure duration. Consequently, the biological effects of the methods also differ (Halgamuge, 2019; Mansourian & Shanei, 2021). This review critically synthesizes evidence from all available methods across in vitro, animal, and clinical studies.

2. Pulsed Electromagnetic Fields (PEMF)

2.1. Clinical Evidence in Postmenopausal Women and Elderly Populations

The earliest controlled clinical observation of PEMF on BMD was reported by Tabrah et al. (1990). In the original study, 20 postmenopausal women (aged 57–75 years) were exposed to a 72 Hz PEMF with a quasirectangular waveform for 10 hours daily over 12 weeks. Bone mineral density of the exposed midshaft radius increased significantly during the treatment period compared to the unexposed contralateral arm. However, this benefit was not sustained: BMD returned toward baseline during the subsequent 36-week observation period, and

at an eight-year follow-up, no significant long-term difference was detected between exposed and unexposed limbs (Tabrah et al., 1998).

Giordano et al. (2001) conducted a single-blind, randomized study comparing 100 Hz PEMF application (60 minutes per day, 3 times a week, for 3 months) with sham application in 40 postmenopausal women with established osteoporosis. At the end of treatment, no significant increase in lumbar spine or femoral neck BMD was observed in either group. However, significant increases in serum osteocalcin and type I procollagen C-terminal propeptide (PICP) levels were observed in the PEMF group during treatment. This dissociation between biochemical markers of bone formation and measurable changes in bone mineral density (BMD) is a significant finding: PEMF may transiently activate bone formation pathways over a 3-month period without producing a measurable densitometric increase (Giordano et al., 2001).

In a study, Liu et al. (2013) assigned 41 postmenopausal women with osteoporosis to either a 30-session PEMF regimen (8 Hz, 3.82 mT, 40 minutes daily) or weekly alendronate (70 mg) treatment. Analyses revealed no statistically significant differences between the groups regarding lumbar spine or proximal femur bone mineral density (BMD) values, muscle strength, or balance scores at weeks 5, 12, or 24. The study concluded that PEMF was non-inferior to alendronate over the 24-week follow-up period (Liu et al., 2013).

Li et al. (2018) randomized 61 postmenopausal women with osteopenia to either a PEMF group (XT-2000B device; 40 minutes per session, 3 sessions per week, for 6 months) or a placebo group, with dual-energy X-ray absorptiometry (DXA) used to assess outcomes. At the end of six months, lumbar spine and hip BMD (bone mineral density) values in the PEMF group increased by 1.46% to 2.04% ($P < 0.05$), bone-specific alkaline phosphatase levels rose by 3.23%, and C-terminal telopeptide of type I collagen levels decreased by 9.12%; conversely, changes in the placebo group were not statistically significant. Vertebral bone marrow fat fraction in the PEMF group decreased significantly by 4.81%, thereby providing the first in vivo human evidence that PEMF can shift mesenchymal stem cell (MSC) differentiation away from adipogenesis and toward osteoblastogenesis Li et al., 2018).

Shanb et al. (2012) compared pulsed electromagnetic field (PEMF) therapy (33 Hz, 50 gauss, 50 minutes per session, 3 sessions per week, for 3 months) with active exercise (treadmill walking and hip and back exercises) in 30 elderly women aged 60–70 years with osteoporosis. Significant increases in bone mineral density (BMD) at the femoral neck and lumbar spine (L3–L5) were observed in both groups, with no significant difference between groups (Shanb et al., 2018).

2.2. PEMF in Special Populations

El-Bandrawy et al. (2018) investigated the effect of PEMF on 40 female athletes aged 15–25 with low bone mineral density (BMD). They compared PEMF therapy—administered at 50 gauss and 33 Hz (for 30 minutes, three times a week for 4 months)—with a control group that received only prescribed calcium and vitamin D supplements. Significant increases in BMD were observed in the femoral neck and lumbar spine (L1–4) regions in both groups. This study demonstrated the efficacy of PEMF in a young population. (El-Bandrawy et al., 2018).

Rakočević Hrnjak et al. (2018) conducted a 3-year prospective clinical study involving 151 participants. In the PEMF group (n = 54, who completed the study), significant improvements were observed in Bone Mineral Density (BMD), T-score, and Z-score values at both the lumbar spine and femoral neck levels in both genders. Conversely, significant deterioration in BMD values was detected in the control group (n = 70, who completed the study). This study represents the longest-duration human PEMF study identified in the literature review and provides strong longitudinal evidence regarding a high-risk population for whom pharmacological options are extremely limited. (Rakočević et al., 2018).

2.3. PEMF in Animal Models: Positive Findings

Rubin et al. (1989) utilized a functionally isolated turkey ulna model to demonstrate the dose-response relationship between PEMF (Pulsed Electromagnetic Field)-induced electrical power and the osteogenic response. Following an eight-week period of disuse (during which 13.0% bone loss was observed in the control group), PEMF applied for one hour daily prevented bone loss; peak efficacy was achieved at an induced power level of 0.01–0.04 $tesla^2$/second. It was observed that effects diminished outside this range (either below or above it), thereby establishing, for the first time, a precise and effective parameter range for osteogenic EMF applications (Rubin et al., 1989).

Shen and Zhao (2010) used a rat hindlimb immobilization model in their study on disuse osteoporosis. Eighty female Sprague-Dawley rats were divided into control, disuse osteoporosis (DOP), and PEMF groups. PEMF treatment was administered for 2 hours immediately after surgery and subsequently once daily. At the end of eight weeks, the proximal femur bone mineral density (BMD) was significantly higher in the PEMF group than in the DOP group (Shen & Zhao, 2010).

2.4. PEMF in Animal Models: Null and Contradictory Findings

Van der Jagt et al. (2014) investigated the effects of locally applied EMF (20 Gauss)—administered for 3 hours a day, 5 days a week over a 6-week period—on bone architecture in ovariectomized (OVX) and sham-OVX rats using in vivo micro-CT. No significant changes were detected in either group at week 3 or

week 6 regarding trabecular bone volume fraction, connectivity density, structure model index, cortical bone volume, or cortical thickness. (Van der Jagt et al., 2014).

Aksoy et al. (2017) induced osteoporosis in male rats via heparin administration and applied PEMF at 0.8 mT and 7.3 Hz for one hour daily over a period of four weeks. This study investigated osteoporosis in males and did not observe a significant improvement in bone mineral density (BMD) with PEMF treatment unlike the majority of published studies utilizing female or ovariectomized animal models, It was suggested that the etiology of osteoporosis and the underlying biological mechanisms play a significant role in determining whether PEMF produces measurable effects (Aksoy et al., 2017).

3. Cellular Mechanisms of Pemf Action on Bone

The mechanistic basis of the effects of EMF on bone has been extensively studied in vitro. Luben et al. (1982) cultured osteoblast-like MMB-1 mouse bone cells in the presence of two different pulsed EMF waveforms used in clinical practice (72 Hz continuous pulse trains and 15 Hz repetitive bursts). Both types of fields significantly reduced cellular cAMP production in response to parathyroid hormone (PTH) and osteoclast-activating factor, without altering basal adenylate cyclase activity. Furthermore, PEMF prevented the PTH-mediated inhibition of collagen synthesis. These findings indicated that the primary site of EMF action is the plasma membrane, even at extracellular field strengths as low as 1 mV/cm (Luben et al., 1982).

As comprehensively discussed by Zhu et al. (2017), the Wnt/β-catenin signaling pathway has emerged as a central mediator of the anabolic effects of PEMF on bone. Notably, Zhu et al. (2017) reported that PEMF mitigates the deterioration of bone microarchitecture and strength by activating the Wnt/LRP5/β-catenin signaling pathway, thereby highlighting a pathway-specific mechanism of action. Numerous animal studies compiled by Zhu et al. (2017) and Wang et al. (2016) have demonstrated that PEMF significantly increases osteoprotegerin (OPG) expression while reducing RANKL levels in osteoblasts. In a rat model of disuse-induced osteoporosis, Shen and Zhao (2010) independently confirmed that PEMF suppresses IL-6, a key upstream stimulator of RANKL. As reported by Rakočević Hrnjak et al. (2018), PEMF appears to upregulate adenosine A2a receptors in osteoclasts, leading to reduced lysosomal enzyme activity and decreased resorption capacity. Luben et al. (1982) reported that PEMF blocks the stimulatory effect of osteoclast-activating factor on osteoblast-like cells.

In a review, Zhang et al. (2020) systematically summarized the effective parameter ranges for various EMF modalities acting on bone cells. Regarding osteoblast proliferation, PEMF demonstrated the greatest effect at frequencies of 7.5–15 Hz or 50–75 Hz and intensities of 0.40–1.55 mT or 3.8–4 mT,

respectively. Sinusoidal EMF with a frequency of 45–60 Hz and an intensity of 0.9–4.8 mT promoted osteoblast differentiation and maturation. Static magnetic fields with intensities of 0.1–0.4 mT or 400 mT also promoted osteoblast differentiation (Zhang et al., 2020). (Zhang et al., 2020).

Kaivosoja et al. (2015) investigated whether PEMF could promote osteoinduction in human bone marrow-derived mesenchymal stem cells (MSCs) in vitro. PEMF increased cell viability in both MSCs and osteoblastic SaOS-2 cells; however, it did not significantly enhance osteoinduction at the low seeding density tested (3,000 cells/cm²) in the absence of additional induction factors (Kaivosoja et al., 2015).

In their reviews, Wang et al. (2016) and Di Bartolomeo et al. (2022) concluded that, although consistent evidence in the literature indicates a beneficial role for PEMF, significant variability in frequency, duration, and amplitude parameters—combined with heterogeneity in applied protocols and outcome measures—makes it difficult to draw definitive conclusions regarding clinical efficacy (Wang et al., 2016; Di Bartolomeo et al., 2022).

4. Radiofrequency EMF (RF-EMF): Effects of Mobile Phone-Frequency Radiation

4.1. Animal Studies

Yıldız et al. (2006) exposed 48 male Sprague-Dawley rats to 900 MHz or 1800 MHz continuous-wave RF-EMF via whole-body exposure for 30 minutes daily over 28 days. Bone Mineral Density (BMD) measurements in the lumbar spine and femoral diaphysis regions showed decreases in both exposure groups compared with the control group; however, these differences were not statistically significant (Yıldız et al., 2006).

Cicek et al. (2009) evaluated biomechanical bone quality rather than Bone Mineral Density (BMD) using a Sprague-Dawley rat model and an exposure system. The researchers found that femoral fracture force, bending resistance, and total fracture energy were significantly reduced ($P < 0.05$) in both the 900 MHz and 1800 MHz groups compared to the control group. These findings suggest that RF-EMF at GSM frequencies may impair the mechanical competence of bone, raising the possibility that structural integrity could be compromised before densitometric changes become detectable (Cicek et al., 2009).

Atay et al. (2009b) exposed 30 male Wistar-Albino rats to 1800 MHz EMF (30 minutes/day, 5 days/week, for 4 weeks) and measured whole-body, lumbar, and femoral bone mineral density (BMD) using the DXA method. In the exposed group, a slight increase in lumbar BMD and a slight decrease in femoral BMD were observed; however, no statistically significant differences were found. In a study, Aslan et al. (2014) specifically investigated the effect of 1800 MHz EMF on fracture healing in 30 Wistar rats with tibial fractures. Radiological,

histological, and mechanical scores were lower in the exposed group than in the control group, though not statistically significant. This led to the conclusion that 1800 MHz EMF has no significant effect on bone fracture healing (Aslan et al., 2014).

4.2. Human Observational Studies

Atay et al. (2009a) examined 150 male volunteers (mean age 31.85) who habitually carried mobile phones on their iliac crest and compared—using the DXA method—the bone mineral density (BMD) of the iliac wing on the side where the phone was carried with that of the contralateral side (unexposed to the phone). The average carrying duration was 14.7 hours per day over a mean period of 6.2 years. DXA values on the side where the phone was carried were slightly lower, but the difference was not statistically significant (Atay et al., 2009a).

In a more comprehensive cross-sectional study, Cidem et al. (2012) included 263 healthy individuals aged 20 to 35 (86 male and 97 female mobile phone users, and 14 male and 66 female non-users). Ultradistal (UD) radius BMD values measured by DXA were significantly lower in mobile phone users than in non-users. This study is the largest observational study in the literature examining the relationship between RF-EMF and BMD and presents the strongest human data on a potential adverse effect (Cidem et al., 2012).

5. Pulsed Electrical Field Stimulation

Lirani-Galvão et al. (2006) investigated the effect of a low-intensity, pulsed electric field (1.5 MHz, 30 mW/cm²) on bone mineral density (BMD) in female Wistar rats. Thirty rats were divided into sham surgery, ovariectomy (OVX), and OVX plus electrical stimulation (OVX+ES) groups. The OVX+ES group received a total of 55 treatment sessions—consisting of 20-minute sessions, five days a week—starting seven days after surgery. Eighty-four days post-surgery, the OVX+ES group exhibited significantly higher overall, spinal, and limb BMD values compared to the OVX group. This study demonstrates that pulsed electric field stimulation at MHz frequencies can prevent estrogen-deficiency-induced bone loss in rats (Lirani-Galvão et al., 2006).

6. Comparative Analysis Across EMF Modalities and Study Levels

6.1. Dose-Response Patterns and Parameter Sensitivity

In the literature regarding the relationship between EMF (electromagnetic fields) and bone, the most direct evidence of a dose-response relationship was presented by Rubin et al. (1989), who demonstrated a distinct inverted-U relationship between induced electrical power and the osteogenic response in a turkey ulna model within the range of 0.01 to 0.04 tesla²/second (Rubin et al., 1989). Similarly, Zhang et al. (2020) summarized various effective frequency ranges applicable to different bone cell types and biological outcomes. Each of

the clinical studies addressed in this review utilized a single set of exposure parameters without making a systematic comparison between different frequency or intensity levels (Zhang et al., 2020). Di Bartolomeo et al. (2022) emphasize that the significant variability in selected parameters and the heterogeneity of protocols across published animal studies severely limit cross-study comparisons and the development of clinical guidelines for practical application (Di Bartolomeo et al., 2022).

6.2. Discrepancies Between Mechanistic and Clinical Evidence

A consistent finding across the literature is that mechanistic evidence derived from in vitro and animal studies is far more positive and consistent than clinical evidence. In vitro studies reliably demonstrate that EMF modulates osteoblast and osteoclast activity (Luben et al., 1982; Zhu et al., 2017; Zhang et al., 2020). With few notable exceptions, animal studies indicate that Bone Mineral Density (BMD) is preserved or improved in most cases (Van der Jagt et al., 2014; Aksoy et al., 2017). In contrast, clinical studies generally report only small-scale improvements in BMD, borderline statistical significance, or equivalence rather than superiority compared with established treatments (Giordano et al., 2001; Liu et al., 2013). This translational gap between preclinical and clinical stages is a common phenomenon in physical therapy research. It reflects factors such as the greater sensitivity of rodent bones to EMF stimulation and the challenges involved in standardizing PEMF application in clinical settings.

6.3. Population-Specific Responses

Existing evidence suggests that the efficacy of PEMF may depend on the population studied. Positive results regarding bone mineral density (BMD) have been most consistently observed in postmenopausal women (Tabrah et al., 1998; Li et al., 2018), elderly women with osteoporosis (Shanb et al., 2012), and female athletes with low BMD (El-Bandrawy et al., 2018; Rakočević Hrnjak et al., 2018). In contrast, Aksoy et al. (2017) did not detect a significant PEMF effect in male rats with heparin-induced osteoporosis, suggesting that estrogen exposure might be necessary for bone responsiveness to PEMF. This hypothesis has not yet been confirmed in human studies and warrants direct investigation.

7. Critical Appraisal of The Evidence Base

The overall quality of evidence in the EMF-BMD literature is significantly limited by various recurring methodological shortcomings. First, sample sizes are invariably small: most human clinical studies included between 30 and 61 participants, while the largest study (Rakočević Hrnjak et al., 2018) involved 124 participants who completed the process under a non-randomized design. None of the studies possessed the statistical power to detect differences in fracture rates, which constitute the primary clinical endpoint in osteoporosis research. Second, follow-up durations are largely inadequate. In most human studies, participants

were followed for 3 to 6 months; a 3-year follow-up period was achieved only in the ESRD study conducted by Rakočević Hrnjak et al. (2018). Third, blinding methods in several studies are inadequate (Giordano et al. 2001; Shanb et al., 2012; El-Bandrawy et al., 2018). Fourth, EMF exposure parameters vary dramatically across studies — from 8 Hz to 100 Hz frequency, from 2 mT to 20 gauss intensity, and from 20 to 120 minutes per session — preventing meaningful cross-study comparison or meta-analysis with clinical confidence (Zhang et al., 2020; Di Bartolomeo et al., 2022). Fifth, the nearly universal use of DXA-measured BMD as the primary outcome may not capture early or partial effects on bone microarchitecture or tissue quality, as demonstrated by the contrast between Cicek et al.'s (2009) finding of impaired biomechanical properties in the absence of significant BMD reduction with RF-EMF exposure (Cicek et al., 2009).

The RF-EMF literature faces additional limitations specific to human observational studies. Atay et al. (2009a) enrolled exclusively male volunteers of working age, limiting generalizability to populations at risk for osteoporosis. Null results, as reported by Van der Jagt et al. (2014), Aksoy et al. (2017), Yildiz et al. (2006), and Atay et al. (2009b), deserve equal scientific weight and collectively suggest that PEMF and RF-EMF effects on bone are neither universal nor robust across experimental conditions (Yildiz et al., 2006; Atay et al., 2009a; Atay et al., 2009a; Cidem et al. (2012).

Although the mechanistic literature is more consistent, it largely operates at the cellular or molecular level and lacks direct clinical validation. Key mechanistic data have been derived from in vivo studies on rodents and human cell lines, rather than from tissue biopsies or molecular analyses obtained from clinical trial participants. (Luben et al., 1982; Zhu et al., 2017; Wang et al., 2016).

8. Research Gaps and Future Directions

Several critical knowledge gaps must be addressed before EMF therapy can be considered evidence-based for BMD management. The most fundamental need is for adequately powered, randomized, double-blind, placebo-controlled trials with fracture prevention as the primary endpoint and at least 2–3 years of follow-up. Optimal exposure parameters — frequency, intensity, waveform, session duration, and total treatment duration — must be systematically evaluated in dose-finding trials before large efficacy studies proceed; applying the dose-response principle established by Rubin et al. (1989) in animals to human clinical protocols is a logical but as yet unvalidated translational step (Rubin et al., 1989). Future studies should differentiate between EMF effects in populations stratified by sex, menopausal status, osteoporosis etiology, and comorbidity, given the evidence that hormonal and disease context may modulate responsiveness (Aksoy et al., 2017).

Molecular biomarkers and advanced imaging endpoints (high-resolution peripheral quantitative CT, MR spectroscopy) should supplement DXA to capture bone quality dimensions beyond density, as pioneered by Li et al. (2018). For RF-EMF, prospective cohort studies with robust exposure assessment and covariate control are needed to resolve the observational signal identified by Cidem et al. (2012). Safety concerns raised by Zhu et al. (2017) require formal long-term safety surveillance in clinical PEMF trials (Cidem et al., 2012; Zhu et al., 2017; Li et al., 2018).

9. Conclusion

Current evidence derived from 28 sources—encompassing in vitro models, animal experiments, and human clinical studies—supports the following conclusions, classified by level of evidence: PEMF demonstrates the strongest evidence for a positive effect on Bone Mineral Density (BMD), particularly in postmenopausal women and elderly women with established osteoporosis (Luben et al., 1982; Tabrah et al., 1998; Zhu et al., 2017; Shen & Zhao, 2010; Li et al., 2018; Shanb et al., 2012; Rakočević Hrnjak et al., 2018). However, confidence in this evidence is at most moderate: it is based on small, short-duration trials with methodological limitations, without fracture endpoint data, and is qualified by reproducible null findings in animal models that suggest parameter sensitivity and population specificity as critical unresolved variables (Van der Jagt et al., 2014; Aksoy et al., 2017).

In rodent models, RF-EMF in the 900–1800 MHz range shows a consistent—though largely not statistically significant—trend toward deterioration in bone mineral density (BMD) and bone mechanical properties (Yildiz et al., 2006; Cicek et al., 2009). One observational study has linked it to forearm osteopenia in female mobile phone users (Cidem et al., 2012); however, this does not constitute sufficient evidence of harm to warrant changing current exposure guidelines. Pulsed electrical field stimulation and static magnetic fields have demonstrated preclinical plausibility without meaningful clinical translation (Lirani-Galvão et al., 2006). In aggregate, PEMF may hold genuine promise as a non-pharmacological adjunct for bone loss prevention and treatment, but the current evidence base is insufficient to support clinical guidelines or widespread implementation. Only through rigorously designed, adequately powered, long-term randomized controlled trials with standardized protocols will the therapeutic position of EMF in bone medicine be definitively established.

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CHAPTER 19

Major Depressive Disorder and Oxidative Stress: General Framework

Hatice Solak[1]

[1] Doç. Dr., Department of Physiology, Faculty of Medicine, Kütahya Health Sciences University

Major depressive disorder (MDD) is a complex psychiatric disorder characterized by considerable heterogeneity in clinical presentation, disease course, and treatment response. Its pathophysiology cannot be explained by a single mechanism; rather, several interacting biological processes, including neuroinflammation, dysregulation of the hypothalamic–pituitary–adrenal axis, mitochondrial dysfunction, and oxidative stress, are thought to contribute alongside alterations in monoaminergic neurotransmission. In recent years, increasing attention has been directed toward the potential contribution of impaired redox homeostasis to the biological basis of depression [1,2].

Oxidative stress arises when the balance between the production of reactive oxygen and nitrogen species and antioxidant defense mechanisms shifts in favor of oxidants. At physiological concentrations, reactive oxygen species participate in cellular signaling and metabolic regulation; however, excessive production or insufficient antioxidant capacity may result in oxidative damage to lipids, proteins, and nucleic acids. The high oxygen consumption and lipid-rich composition of the brain, together with its susceptibility to oxidative injury, make the central nervous system particularly vulnerable to disturbances in redox homeostasis [1].

Evidence supporting increased oxidative stress in depression extends beyond individual studies. In a meta-analysis of 23 studies including 4,980 participants, Palta et al. reported that depression was associated with higher oxidative stress and lower antioxidant status [2]. A larger meta-analysis by Liu et al., including 115 studies, showed reduced total antioxidant capacity during acute depressive episodes together with increased levels of oxidative damage products such as malondialdehyde and 8-F2-isoprostanes [3]. Similarly, Black et al. demonstrated significantly increased levels of both 8-OHdG and F2-isoprostanes in depression [4]. Nevertheless, substantial heterogeneity across studies, including differences in biological matrices, analytical methods, disease stage, and treatment status, suggests that oxidative stress should not be interpreted as a simple unidirectional cause of depression.

1.Biological Basis of Oxidative Stress

Oxidative stress develops when the balance between reactive oxygen species (ROS) and reactive nitrogen species (RNS) generated during cellular metabolism and the antioxidant systems that limit their effects is disrupted. Superoxide anion, hydrogen peroxide, and hydroxyl radicals are among the major ROS, whereas nitric oxide and peroxynitrite are important components of nitrosative stress. At physiological concentrations, these molecules participate in intracellular signaling and redox regulation; however, excessive production or insufficient antioxidant defense may lead to structural and functional damage to lipids, proteins, and nucleic acids. The high oxygen consumption and lipid-rich

composition of the brain make the central nervous system particularly susceptible to oxidative injury [5,6].

Mitochondria have a central role not only in cellular energy production but also in maintaining redox homeostasis. Although a certain amount of ROS is physiologically generated during electron transport, mitochondrial dysfunction may increase electron leakage and ROS production. The resulting oxidative damage can affect mitochondrial proteins, lipids, and DNA, further impairing energy production and creating a self-perpetuating cycle. Clinical and experimental evidence suggests that MDD and chronic stress are associated with alterations in mitochondrial biogenesis, redox imbalance, oxidative damage to cellular macromolecules, and apoptotic processes. Nevertheless, findings are not entirely consistent across studies, and mitochondrial dysfunction cannot yet be considered an isolated causal mechanism of depression [6,7].

Another important source of ROS is the nicotinamide adenine dinucleotide phosphate oxidase (NOX) enzyme family. Unlike mitochondria, one of the principal functions of NOX enzymes is the direct generation of ROS. Increased NOX activity in neurons, microglia, and vascular endothelial cells may contribute to the interaction between oxidative stress and inflammatory responses. In recent years, the NOX system has also gained attention in depression, as enhanced NOX activation has been linked to increased ROS production, neuroinflammation, and cellular dysfunction. However, because human evidence remains limited, the value of NOX enzymes as biomarkers or therapeutic targets in depression is still under investigation [8].

Enzymatic and non-enzymatic antioxidant systems act together to control oxidant production. Superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPx) constitute major enzymatic defense mechanisms, whereas glutathione, uric acid, tocopherols, and other low-molecular-weight antioxidants contribute to redox homeostasis. Although several studies suggest impaired antioxidant defense in depression, findings regarding SOD and GPx activity may vary according to disease stage, biological matrix, and treatment status. Increasing attention is therefore being given to the bidirectional interaction among oxidant production, mitochondrial dysfunction, and inflammation. Mitochondria-derived ROS may activate inflammatory signaling pathways, whereas pro-inflammatory cytokines can impair mitochondrial function and redox balance, thereby contributing to the persistence of oxidative stress [7,9].

2.Oxidative Stress Biomarkers in Depression

Total antioxidant capacity (TAC), total oxidant status (TOS), and oxidative stress index (OSI) are commonly used to assess overall redox balance in depression. Patients with MDD have been reported to show lower TAC and higher TOS and OSI levels, with these alterations also being associated with

depression severity [10]. Meta-analytic evidence further supports reduced antioxidant capacity during acute depressive episodes [3].

MDA, F2-isoprostanes, and 8-OHdG are among the most frequently studied markers of oxidative damage. Meta-analyses have demonstrated increased MDA, a marker of lipid peroxidation, in depression [11], together with higher levels of F2-isoprostanes and 8-OHdG, a marker of oxidative DNA damage [12]. These findings suggest that both lipids and nucleic acids may be affected by oxidative injury in depression.

More recently, biomarkers such as NOX1, Raftlin, and oxLDL have gained attention. Increased NOX1 and Raftlin levels have been reported in treatment-naïve patients with first-episode MDD [13]. Similarly, serum oxLDL levels were found to be higher in drug-naïve patients with MDD than in healthy controls [14]. Although oxLDL can enhance ROS production and inflammatory signaling through LOX-1, the clinical biomarker value of LOX-1 in MDD remains insufficiently studied.

3. Oxidative Stress Biomarkers and Treatment Response

The potential value of oxidative stress biomarkers in predicting treatment response has been investigated. Lindqvist et al. reported that higher baseline F2-isoprostane levels were associated with poorer response to SSRI treatment [15]. In contrast, another follow-up study found higher baseline F2-isoprostane levels in treatment responders [16]. These conflicting findings indicate that a single oxidative stress marker is not yet sufficient to predict treatment response.

Some oxidative stress markers may improve following antidepressant treatment. A recent review reported a general normalization of oxidative stress markers during remission [1]. However, larger longitudinal studies are still needed before these biomarkers can be incorporated into routine clinical practice.

4. Clinical Potential and Conclusion

Current evidence indicates increased oxidative stress and impaired antioxidant defense in depression. However, biomarkers such as TAC, TOS, MDA, F2-isoprostanes, 8-OHdG, NOX1, and oxLDL do not have sufficient diagnostic value when considered individually. Given the biological heterogeneity of MDD, multimarker approaches combining oxidative stress markers with inflammatory and clinical variables may provide greater clinical value [3,1].

Larger longitudinal studies using standardized methodologies are needed to determine whether oxidative stress biomarkers can contribute to identifying biological subtypes of depression, assessing disease severity, or predicting treatment response. Further advances in this field may allow oxidative stress to be considered not only as a pathophysiological mechanism but also as a biological target relevant to future personalized treatment strategies.

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CHAPTER 20

Comparison of Breast-Conserving Surgery and Modified Radical Mastectomy Following Primary Chemotherapy in Breast Cancer: A Retrospective Cohort Study

Alper Çalışkan[1]

[1] Op. Dr., Selahaddin Eyyubi State Hospital, Orcid:0000-0001-7527-3255

INTRODUCTION

Breast cancer is the most common malignant neoplasm affecting women worldwide and the leading cause of cancer-related mortality in this population. Approximately 180,000 new cases are diagnosed annually in the United States, with breast cancer ranking second only to lung cancer as a cause of cancer death in women [1]. In Turkey, epidemiological data show increasing incidence, with breast cancer being the most frequent malignancy among women in western provinces [2].

Historically, the surgical management of breast cancer was dominated by the Halsted radical mastectomy. Progressive refinements led to the modified radical mastectomy (MRM), which reduced operative morbidity while maintaining locoregional control [3]. From the 1980s onwards, landmark randomised controlled trials conducted by the National Surgical Adjuvant Breast and Bowel Project (NSABP), the European Organisation for Research and Treatment of Cancer (EORTC), and the Milan group established that breast-conserving surgery (BCS) combined with whole-breast irradiation was oncologically equivalent to mastectomy for early-stage disease [3–7].

Primary (neoadjuvant) chemotherapy delivers systemic treatment prior to surgery and has further expanded BCS eligibility by achieving tumour downstaging in patients who would otherwise require mastectomy [8]. It additionally permits in vivo assessment of chemosensitivity and provides pathological complete response (pCR) as a surrogate endpoint for long-term outcome [9].

The aim of this study was to compare OS, DFS, and local recurrence rates in breast cancer patients who underwent BCS versus MRM following primary chemotherapy at Tepecik Training and Research Hospital over a ten-year period (1994–2004).

METHOD

Study Design and Patient Selection

This retrospective cohort study included 159 patients with histologically confirmed breast cancer treated between January 1994 and December 2004. Patients had received primary chemotherapy followed by curative surgery (BCS or MRM), and were staged according to the AJCC 6th edition. Patients with distant metastatic disease, bilateral breast cancer, or incomplete records were excluded. The study was conducted in accordance with the Declaration of Helsinki, with institutional ethics committee approval.

Primary Chemotherapy

All patients received anthracycline-based primary chemotherapy prior to surgery, most commonly cyclophosphamide, epirubicin, and fluorouracil (CEF).

Tumour response was assessed clinically and radiologically after three to four cycles. pCR was defined as the absence of invasive carcinoma in the breast and axillary lymph nodes on final histopathological examination.

Surgical Procedures

BCS comprised wide local excision with a minimum 2 mm tumour-free margin and level I–II axillary lymph node dissection. MRM (Patey or Auchincloss-Madden technique) involved removal of the entire breast with level I–III axillary clearance. The surgical approach was determined in accordance with tumour response, residual tumour size, multifocality, and patient preference after informed consent.

Adjuvant Treatment

All BCS patients received postoperative radiotherapy to the whole breast (45–50 Gy in 25 fractions, 1.8–2 Gy/fraction, over 5–6 weeks), with a tumour-bed boost of 10–16 Gy where indicated. Hormone receptor-positive patients received adjuvant tamoxifen 20 mg/day for five years.

Follow-up and Outcomes

Follow-up was performed at three-month intervals for the first two years, six-monthly for the next three years, then annually. OS was defined as time from surgery to death from any cause; DFS as time to first event (local recurrence, distant metastasis, or death).

Statistical Analysis

Categorical variables were compared using the Pearson chi-square or Fisher's exact test. Survival curves were constructed by the Kaplan–Meier method and compared with the log-rank test. A p-value <0.05 was considered statistically significant. Analyses were performed using SPSS v15.0.

RESULTS

Patient Characteristics

The two groups were well balanced. Most patients were aged 41–50 years (BCS 41.0%, MRM 33.3%). Invasive ductal carcinoma was the predominant histological subtype (BCS 78.1%, MRM 75.9%). Oestrogen receptor positivity was present in 58.1% (BCS) and 59.3% (MRM) of patients. No significant intergroup differences were observed in age, stage, histology, or hormone receptor status (Table 1).

Table 1. Baseline characteristics

Characteristic	BCS (n=105)	MRM (n=54)
Age group (years)		
≤40	18 (17.1%)	9 (16.7%)
41–50	43 (41.0%)	18 (33.3%)
51–60	31 (29.5%)	19 (35.2%)
>60	13 (12.4%)	8 (14.8%)
Clinical stage		
Stage I	11 (10.5%)	5 (9.3%)
Stage IIA	42 (40.0%)	21 (38.9%)
Stage IIB	31 (29.5%)	15 (27.8%)
Stage IIIA	15 (14.3%)	9 (16.7%)
Stage IIIB	6 (5.7%)	4 (7.4%)
Histological type		
Invasive ductal carcinoma	82 (78.1%)	41 (75.9%)
Invasive lobular carcinoma	15 (14.3%)	8 (14.8%)
Other	8 (7.6%)	5 (9.3%)
ER status		
Positive	61 (58.1%)	32 (59.3%)
Negative	44 (41.9%)	22 (40.7%)
pCR to primary chemotherapy	11 (10.5%)	5 (9.3%)

BCS: Breast-conserving surgery; MRM: Modified radical mastectomy; ER: Oestrogen receptor; pCR: Pathological complete response. Values are n (%).

Response to Primary Chemotherapy

The pCR rate was 10.5% in the BCS group and 9.3% in the MRM group ($p>0.05$). Fine needle aspiration biopsy confirmed diagnosis in 94.4% of patients. Tumour downstaging sufficient to permit BCS was achieved in 66% of initially MRM-indicated cases.

Survival

Median follow-up was 72.8 months (BCS) and 91.3 months (MRM). Five-year OS was 83.46% versus 84.62% ($p>0.05$); five-year DFS was 78.37% versus 75.45% ($p>0.05$). Ten-year OS rates were 71.5% and 71.7%, respectively (Table 2).

Table 2. Survival outcomes

Outcome	BCS (n=105)	MRM (n=54)	p-value
5-year overall survival	83.46%	84.62%	>0.05
5-year disease-free survival	78.37%	75.45%	>0.05
10-year overall survival	71.5%	71.7%	>0.05
Local recurrence rate	9.5%	8.3%	>0.05
Median follow-up (months)	72.8	91.3	—
95% CI – OS	0.699–0.845	0.624–0.784	—
95% CI – DFS	0.756–0.907	0.497–0.743	—

OS: Overall survival; DFS: Disease-free survival; CI: Confidence interval

Local Recurrence and Complications

Local recurrence occurred in 9.5% of BCS and 8.3% of MRM patients ($p>0.05$). Late radiotherapy morbidity in the BCS group included radiation pneumonitis (3%), myocarditis (2%), and plexopathy (1%). Axillary lymphoedema was observed in 15.2% of all patients undergoing axillary dissection, with no significant intergroup difference.

DISCUSSION

The results of this study are consistent with the major randomised trials establishing the oncological equivalence of BCS and mastectomy. Our five-year OS rates (BCS 83.46%, MRM 84.62%) align with those of landmark trials including NSABP B-06 [4], EORTC 10801 [7], and the Danish cooperative study [5], and are summarised in Table 3.

Table 3. Comparison with published literature

Study	n	5-yr OS BCS	5-yr OS MRM	p-value
Fisher et al. (NSABP B-06)	1,219	76%	75%	>0.05
Blichert-Toft et al.	905	79%	82%	>0.05
Van Dongen et al. (EORTC)	868	65%	66%	>0.05
Semiglazov et al.	272	81%	79%	>0.05
Present study	159	83.5%	84.6%	>0.05

BCS: Breast-conserving surgery; MRM: Modified radical mastectomy; OS: Overall survival.

Neoadjuvant chemotherapy facilitated BCS in 66% of patients initially requiring mastectomy, consistent with NSABP B-18, which reported a significantly higher BCS rate with primary AC chemotherapy compared with adjuvant treatment [8]. Bonadonna et al. reported pCR rates of 10–20% with anthracycline-based neoadjuvant regimens [9], comparable with the 10.5% observed in our cohort.

Adjuvant tamoxifen reduced contralateral breast cancer incidence and improved DFS in hormone receptor-positive patients, consistent with EBCTCG meta-analyses [10]. The local recurrence rate of 9.5% in the BCS group is within the range reported in comparable series.

The present cohort included 21 patients with Stage IIIA–IIIB disease (BCS: 14.3% Stage IIIA and 5.7% Stage IIIB; MRM: 16.7% and 7.4%, respectively), representing a subgroup in whom primary chemotherapy is particularly valuable. In locally advanced breast cancer (LABC), systemic treatment prior to surgery has been shown to render inoperable tumours resectable and to convert mandatory mastectomy candidates into BCS-eligible patients. The NSABP B-27 trial demonstrated that adding docetaxel to preoperative AC chemotherapy doubled the pCR rate compared with AC alone and significantly increased the proportion of patients eligible for breast conservation [11]. Although our regimen was anthracycline-based without taxane intensification—reflecting the standard of care during the 1994–2004 study period—the 66% conversion rate from MRM indication to BCS eligibility following primary chemotherapy is consistent with results reported in contemporaneous series.

The significance of pathological complete response in our series warrants further comment. The pCR rate of 10.5% (BCS group) and 9.3% (MRM group) is consistent with the 10–20% range reported for anthracycline-based neoadjuvant regimens in the pre-taxane era [9]. A large pooled analysis by Cortazar et al., encompassing data from 12 international trials and over 11,000 patients, established that pCR—defined as the absence of residual invasive disease in the breast and lymph nodes—is strongly associated with improved event-free survival and OS, particularly in triple-negative and HER2-positive subtypes [12]. Although receptor subtype data were not available for systematic classification in the present retrospective cohort, the association between pCR and long-term outcome is consistent with our survival findings: patients achieving pCR in both groups demonstrated markedly favourable clinical courses during follow-up. These observations reinforce the prognostic value of pCR as a surrogate endpoint and the importance of optimising neoadjuvant regimens to maximise pathological response.

Axillary management in both arms of this study consisted of formal level I–III lymph node dissection, reflecting the standard of care throughout the enrolment period. Axillary lymph node status remains the most robust prognostic

determinant in operable breast cancer, and its accurate assessment is critical for staging, adjuvant treatment planning, and locoregional radiotherapy decisions. Sentinel lymph node biopsy (SLNB) was being validated in early-stage breast cancer during the latter portion of our study period, with landmark trials by Veronesi et al. and Krag et al. demonstrating that SLNB could reliably identify nodal status while significantly reducing the morbidity associated with complete axillary dissection [13]. The axillary lymphoedema rate of 15.2% observed in our cohort is consistent with rates reported for ALND in the pre-SLNB era and underscores the clinical value of adopting less morbid axillary staging procedures in appropriately selected patients, particularly those with favourable tumour biology and good response to primary chemotherapy.

The role of postoperative radiotherapy in the BCS arm merits discussion in the context of locoregional control. All BCS patients in our series received whole-breast irradiation at 45–50 Gy, with a tumour-bed boost where indicated. This protocol is consistent with the evidence base established by the EBCTCG overview, which demonstrated that radiotherapy after BCS approximately halved the ten-year local recurrence risk and reduced the fifteen-year breast cancer mortality by approximately one sixth [14]. The low and statistically comparable local recurrence rates in our two groups (BCS: 9.5%, MRM: 8.3%) reflect the effectiveness of this combined surgical and radiotherapeutic strategy. Late radiation-associated morbidity, including the 3% rate of radiation pneumonitis and 2% rate of myocarditis observed in this series, must be weighed against this locoregional benefit, particularly as improvements in radiotherapy planning and delivery—including intensity-modulated radiotherapy (IMRT) and partial breast irradiation techniques—have substantially reduced these late toxicities in contemporary practice.

Limitations include the retrospective design, long enrolment period introducing potential temporal variation in protocols, modest sample size limiting subgroup analyses, and absence of quality-of-life data.

CONCLUSION

BCS following primary chemotherapy provides equivalent OS, DFS, and local recurrence outcomes compared with MRM in appropriately selected breast cancer patients. These findings support BCS as an oncologically safe, organ-preserving alternative to mastectomy. Multidisciplinary assessment and adherence to postoperative radiotherapy are essential for optimal outcomes.

DESCRIPTIONS

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CHAPTER 21

Umbilical Cord Mesenchymal Stem Cell-Derived Exosomes in Type 2 Diabetes: From Mechanism to Therapeutic Application

Muhammet Volkan Bülbül [1]
Secil Nazife Parlak [2]
Merve Nur Güney [3]

[1] Assistant Professor, Agri Ibrahim Cecen University, School of Medicine, Histology and Embryology Department, Agri, Turkiye
[2] Assistant Professor, Agri Ibrahim Cecen University, School of Medicine, Histology and Embryology Department, Agri, Turkiye
[3] Research Assistant, Agri Ibrahim Cecen University, School of Medicine, Histology and Embryology Department, Agri, Turkiye

1. Introduction

T2DM is a major public-health problem of the twenty-first century. Its clinical course reflects the combined effects of peripheral insulin resistance and a gradual loss of pancreatic β-cell function (Abel et al., 2024). Current projections place the global number of people with T2DM at about 783 million by 2045, with substantial consequences for health-care systems (Sun et al., 2022; Zhang et al., 2026). Long-standing hyperglycaemia exposes tissues to oxidative stress, low-grade inflammation, endoplasmic reticulum stress, and mitochondrial dysfunction. These changes help explain the occurrence of microvascular complications, including diabetic nephropathy (DN), retinopathy (DR), and neuropathy, as well as macrovascular disease such as cardiovascular disease (Lu et al., 2024).

Management of T2DM includes lifestyle measures and glucose-lowering drugs. Sodium-glucose cotransporter-2 (SGLT2) inhibitors and glucagon-like peptide-1 (GLP-1) receptor agonists have added cardiovascular and renal benefits to this approach. Their use, however, does not restore failing β-cells or completely prevent the progression of diabetic complications (Młynarska et al., 2025). Approaches that preserve β-cell mass and improve repair in affected organs are therefore still required.

MSCs are of interest in regenerative medicine because they can self-renew, differentiate along several lineages, and regulate immune responses. Human umbilical cord-derived MSCs (hUCMSCs) are especially practical in this regard. The umbilical cord is normally discarded after birth, so it provides an accessible source without the ethical concerns associated with some other cell sources. Compared with adult tissue-derived MSCs, hUCMSCs have a more primitive phenotype, greater proliferative capacity, a strong paracrine secretome, and lower immunogenicity (Mebarki et al., 2021). Although hUCMSC transplantation has shown potential in T2DM, poor engraftment, possible tumorigenicity, and microvascular occlusion remain important limitations.

The benefit observed after MSC administration is now thought to depend largely on paracrine signalling. Extracellular vesicles (EVs), particularly exosomes, appear to mediate a substantial part of this signalling (Jiao et al., 2024). Exosomes are membrane-bound vesicles, approximately 30–150 nm in size, released by many cell types. They transfer bioactive cargo to recipient cells and can thereby alter gene expression and cell behaviour. Exosomes released by hUCMSCs (hUCMSCs-Exos) contain miRNAs, mRNAs, proteins, and lipids that are consistent with the regenerative and immunomodulatory properties of the parent cells (Yang et al., 2022).

hUCMSCs-Exos provide a cell-free way of examining MSC-based treatment in T2DM. Their stability, ability to cross biological barriers, and lower risk profile relative to living-cell preparations are potential practical advantages

(Xiong et al., 2021). Preclinical studies published over the past five years report improved insulin sensitivity, protection of pancreatic β-cells from inflammatory and apoptotic injury, and reduced tissue damage in several diabetic complications (Tu et al., 2026). The following sections examine these findings, the mechanisms proposed to account for them, and their implications for later clinical work.

2. Characteristics and Biogenesis of hUCMSCs-Exos

2.1. Biogenesis and Secretion

Exosomes are extracellular vesicles that arise from the endosomal pathway. Their formation begins with plasma-membrane invagination and the generation of early endosomes. As these structures mature, the limiting membrane buds inward and produces intraluminal vesicles (ILVs). A late endosome containing ILVs is termed a multivesicular body (MVB) (Hade et al., 2021).

Cargo selection during ILV formation relies on more than one mechanism. The endosomal sorting complex required for transport (ESCRT), composed of ESCRT-0, -I, -II, and -III, has a central role. Tetraspanins, ceramide, and lipid rafts provide ESCRT-independent routes for sorting cargo and forming ILVs (Isaac et al., 2021). An MVB may subsequently fuse with a lysosome, leading to degradation of its contents, or merge with the plasma membrane and release ILVs as exosomes. Rab GTPases, especially Rab27a and Rab27b, SNARE proteins, and intracellular calcium help control the latter event (Xu et al., 2023). Figure 1 summarises these steps.

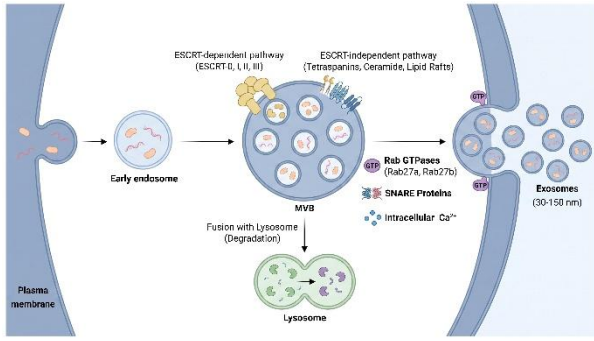


Figure 1. Formation and release of exosomes by mesenchymal stem cells. Plasma-membrane invagination produces early endosomes. A second inward budding of the endosomal limiting membrane generates intraluminal vesicles (ILVs) and forms multivesicular bodies (MVBs). ILV cargo is selected through ESCRT-dependent mechanisms (ESCRT-0, -I, -II, and -III) or ESCRT-independent mechanisms involving tetraspanins, ceramide, and lipid rafts. MVBs can be directed to lysosomes for degradation or can fuse with the plasma membrane, releasing ILVs as exosomes (30–150 nm). Rab27a, Rab27b, SNARE proteins, and intracellular Ca^{2+} regulate release.

2.2. Molecular Composition and Cargo

The activity of hUCMSCs-Exos is linked to the molecules incorporated during vesicle formation. This cargo reflects the condition of the parent hUCMSC. Exosomal membranes contain cholesterol, sphingomyelin, phosphatidylserine, and ceramide. These lipids contribute to membrane stability and facilitate communication with recipient cells by membrane fusion or receptor-mediated endocytosis.

Protein profiling shows that hUCMSCs-Exos contain both conventional exosome markers and proteins with functional relevance. The marker set includes tetraspanins (CD9, CD63, and CD81), heat-shock proteins (HSP70 and HSP90), and proteins involved in transport or membrane fusion, such as Rab GTPases, annexins, and flotillins (Lai et al., 2022). Other identified proteins are associated with immunomodulation, extracellular-matrix (ECM) remodelling, angiogenesis, and cytoprotection.

The nucleic-acid component of hUCMSCs-Exos is also important, particularly the microRNAs (miRNAs). MiRNAs are non-coding RNAs of roughly 22 nucleotides. They bind to 3′-untranslated regions (3′-UTRs) of target mRNAs and can promote mRNA degradation or inhibit translation. Sequencing studies have detected miR-146a-5p, miR-21-5p, miR-100-5p, miR-191-5p, and miR-133a in hUCMSCs-Exos. These miRNAs are linked to pathways that regulate inflammation, apoptosis, fibrosis, and cellular metabolism (He et al., 2021; Khezrian et al., 2025). Their transfer to recipient cells in the diabetic microenvironment is one explanation for the biological effects attributed to hUCMSCs-Exos.

2.3. Advantages of hUCMSCs as an Exosome Source

Bone marrow, adipose tissue, dental pulp, and other tissues can serve as MSC sources for exosome production. hUCMSCs are often favoured because the umbilical cord is available after birth and is otherwise discarded. In comparison with adult tissue-derived MSCs, their neonatal phenotype is associated with stronger proliferation, a more active paracrine profile, and a robust immunomodulatory secretome (Bongso & Fong, 2013; Li et al., 2014; Wang et al., 2025). Reduced MHC class II expression also contributes to the low immunogenicity of hUCMSCs. These properties are reflected in their secreted exosomes and support consideration of allogeneic applications without immunosuppression (Y. Chen et al., 2021). Table 1 sets hUCMSCs-Exos alongside exosomes from other commonly used MSC sources.

Characteristic	hUCMSC-Exos	BM-MSC-Exos	AD-MSC-Exos
Donor source	Discarded perinatal tissue	Bone marrow aspiration (invasive)	Liposuction (minimally invasive)
Ethical concerns	None	Minimal	Minimal
Proliferative capacity of parent cell	High (neonatal phenotype)	Moderate (age-dependent decline)	High
Immunogenicity	Very low	Low	Low
Paracrine potency	High	Moderate to High	Moderate
Allogeneic use	Highly suitable	Suitable	Suitable
Standardization potential	High	Moderate	Moderate

Table 1. Features of exosomes derived from commonly used MSC sources. BM-MSC: bone marrow mesenchymal stem cell; AD-MSC: adipose-derived mesenchymal stem cell.

3. Molecular Mechanisms of hUCMSCs-Exos in Type 2 Diabetes

Peripheral insulin resistance and pancreatic β-cell dysfunction reinforce one another in T2DM. hUCMSCs-Exos have been reported to influence both processes, rather than acting at a single molecular site.

3.1. Reversal of Peripheral Insulin Resistance

Early T2DM is characterised by reduced insulin responsiveness in skeletal muscle, adipose tissue, and the liver. Cytokine-driven inflammatory signalling contributes to this defect. TNF-α, IL-6, and IL-1β activate pathways such as NF-κB and JNK, which promote inhibitory serine phosphorylation of insulin receptor substrate-1 (IRS-1). Once IRS-1 is affected in this way, PI3K/AKT signalling, GLUT4 translocation, and glucose uptake are impaired (Freeman et al., 2023).

Several mechanisms may account for the effects of hUCMSCs-Exos on insulin sensitivity. One concerns macrophage polarisation. In diabetic tissue, resident macrophages commonly display the pro-inflammatory M1 phenotype (Olefsky & Glass, 2010; Xiao et al., 2014; Yin, Hao, Cheng, Gao, et al., 2018; Yin, Hao, Cheng, Zang, et al., 2018). hUCMSCs-Exos can promote a shift towards the M2 phenotype, which is associated with anti-inflammatory and tissue-repair functions. Lower local and systemic inflammation may then reduce cytokine-mediated interference with insulin signalling. Exosomal miRNAs and proteins can also influence cell metabolism. Their delivery has been linked to activation of AMP-activated protein kinase (AMPK), with subsequent improvements in glucose use and mitochondrial function in skeletal muscle and

hepatic cells (M. T. Chen et al., 2021; Kim et al., 2024; Yap et al., 2022). Figure 2 presents these related pathways.

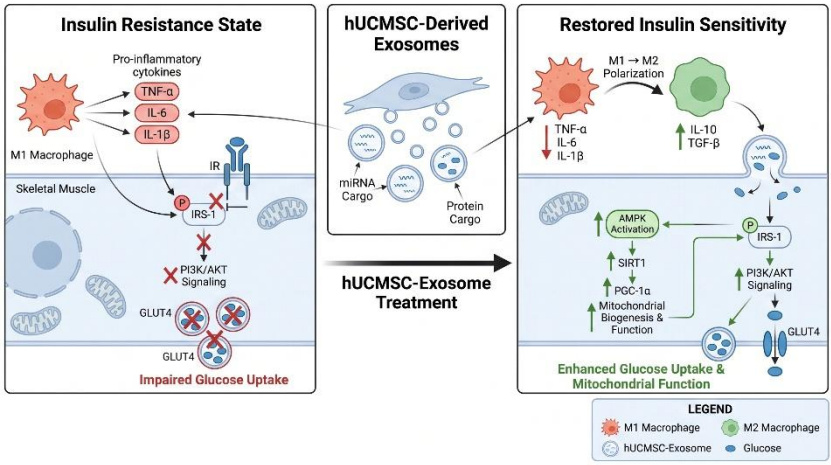


Figure 2. Processes proposed to improve peripheral insulin resistance in skeletal muscle after treatment with hUCMSC-derived exosomes. In the diabetic setting (left), M1 macrophage-derived TNF-α, IL-6, and IL-1β induce inhibitory serine phosphorylation of IRS-1 and disrupt PI3K/AKT signalling and GLUT4 translocation. Following exosome treatment (right), M2 polarisation reduces cytokine-mediated inhibition. In parallel, AMPK activation is linked to the SIRT1/PGC-1α cascade, recovery of IRS-1 activity, reactivation of PI3K/AKT signalling, and GLUT4-mediated glucose uptake. IR, insulin receptor; IRS-1, insulin receptor substrate-1; AMPK, AMP-activated protein kinase; GLUT4, glucose transporter type 4.

3.2. Protection and Restoration of Pancreatic β-Cells

MiR-191-5p transfer is one of the mechanisms described most clearly for hUCMSC-derived extracellular vesicles. This miRNA targets death-associated protein kinase 1 (DAPK1) and activates PI3K/AKT and STAT pathways. Increased expression of Nrf2, SOD1, and Bcl-2 follows, limiting oxidative injury in insulin-responsive tissues and pancreatic β-cells. The reported outcomes include improved insulin sensitivity and better β-cell survival (Li et al., 2025).

Functional β-cell mass declines as T2DM advances. Glucolipotoxicity can induce apoptosis and dedifferentiation, while immune-mediated injury adds to the loss. The result is relative or absolute insulin deficiency. Efforts to retain viable β-cells and recover their function are consequently important. In experimental settings, hUCMSCs-Exos protect β-cells exposed to glucolipotoxic or oxidative stress. After uptake, they provide antioxidant enzymes and regulatory miRNAs. Activation of the Nrf2 pathway can reduce reactive oxygen

species (ROS) accumulation and apoptosis (Li et al., 2025). hUCMSCs-Exos have also been associated with inhibition of NLRP3 inflammasome activation, lower Bax expression, and higher Bcl-2 expression in β-cells.

The exosomal cargo may additionally affect the inflammatory conditions around pancreatic islets. Proteins such as insulin (INS) and superoxide dismutase 1 (SOD1) have been reported to suppress macrophage activation and to lower IL-6, TNF-α, and CCL-2 production (Chen et al., 2025). Factors that promote autophagy may also help remove damaged organelles and misfolded proteins. Together, these effects may support β-cell viability and function.

3.3. Modulation of Key Signaling Pathways

Studies in the diabetic setting identify several recurring molecular targets of hUCMSCs-Exos. Table 2 brings these reported mechanisms and their associated cargo together.

Molecular Mechanism	**Key Cargo**	**Target Pathway / Molecule**	**Therapeutic Effect**	**Reference**
Antioxidant defense activation	miR-191-5p	DAPK1 → PI3K/AKT/Nrf2	Reduces ROS, protects β-cells	(Li et al., 2025)
Immunomodulation (T1DM model)	INS, SOD1 proteins	Macrophage activation suppression	Attenuates systemic inflammation	(Chen et al., 2025)
Macrophage M2 polarization	miR-486-5p	PIK3R1 → PI3K/AKT	Attenuates renal inflammation in DN	(Su et al., 2024)
Anti-fibrotic signaling	miR-133a	TGF-β/Smad pathway	Reduces myocardial fibrosis in DCM	(Liu et al., 2024)
Anti-pyroptotic signaling	miR-100-5p	FOXO3 → NLRP3 inflammasome	Protects cardiomyocytes from pyroptosis	(Liang et al., 2020)
Angiogenesis promotion	VEGF-related proteins	PI3K/AKT	Accelerates diabetic wound closure	(Teng et al., 2022)
Renal Nrf2 activation	Nrf2-activating factors	Nrf2/HO-1 pathway	Reduces oxidative damage in DN	(Zhang et al., 2022)

Table 2. Reported molecular mechanisms and cargo of hUCMSCs-Exos in diabetes. DN: diabetic nephropathy; DCM: diabetic cardiomyopathy; ROS: reactive oxygen species.

4. Therapeutic Applications in Diabetic Complications

Hyperglycaemia affects the microvasculature and macrovasculature over time, producing complications that account for a substantial part of diabetes-related illness and death. hUCMSCs-Exos are being studied in this context because their cargo has anti-inflammatory, angiogenic, and tissue-repair activity.

4.1. Diabetic Nephropathy

Diabetic nephropathy (DN) is a common cause of end-stage renal disease. Its pathological features include podocyte injury, glomerular hypertrophy, mesangial expansion, and tubulointerstitial fibrosis. Oxidative stress, inflammation, and activation of the renin–angiotensin system under hyperglycaemic conditions contribute to these lesions.

In experimental DN, intravenous hUCMSCs-Exos have been associated with better renal function and less structural damage. In one study, exosomes obtained from hUCMSCs exposed to a diabetic microenvironment (Exo-pre) were more protective than control exosomes. They contained more miR-486-5p, which targets PIK3R1, activates PI3K/AKT signalling, promotes M2 polarisation, and reduces renal inflammation (Su et al., 2024). Separate work found that hUCMSC-derived miR-146a-5p favours M2 polarisation through inhibition of TRAF6/STAT1 signalling and is associated with lower inflammatory cytokine levels and less renal fibrosis (Zhang et al., 2022).

An engineered preparation illustrates a further direction. hUCMSCs-Exos loaded with Exendin-4, a GLP-1 receptor agonist, have shown a multimodal effect in DN. The reported mechanism includes induction of CD4+ regulatory T (Treg) cells in association with particular gut-microbiota components. This finding raises the possibility that gut–immune interactions can be incorporated into exosome-based treatment (L. Wang, A. Liang, et al., 2024).

4.2. Diabetic Wound Healing and Foot Ulcers

Diabetes-associated wounds often remain open for prolonged periods and can progress to lower-extremity amputation. Their delayed healing reflects persistent inflammation, inadequate new-vessel formation, poor migration of keratinocytes and fibroblasts, and insufficient ECM remodelling.

Local application of hUCMSCs-Exos has accelerated wound closure in diabetic models. Endothelial cells, fibroblasts, and keratinocytes in the wound bed internalise the vesicles. Increased VEGF expression and PI3K/AKT signalling are associated with endothelial proliferation and tube formation. The vesicles can also favour M2 polarisation and reduce TNF-α, IL-1β, and iNOS, helping to resolve persistent inflammation. During the proliferative phase, enhanced fibroblast migration and collagen deposition have also been reported (Teng et al., 2022).

Delivery materials are being explored to retain exosomes at the wound site and extend their activity. When hUCMSCs were preconditioned with the Wnt/β-catenin agonist CHIR99021, exosome yield increased 1.5-fold. Levels of exosomal proteins related to cell migration and wound healing also increased, and wound closure was faster in diabetic mice (L. Wang, J. Chen, et al., 2024). Self-healing injectable hydrogels offer another option: they retain hUCMSCs-Exos locally, release them over time, and preserve vesicle bioactivity while the wound environment is modified (Zhang et al., 2025).

4.3. Diabetic Cardiomyopathy

Diabetic cardiomyopathy (DCM) describes cardiac structural and functional changes in diabetes that arise independently of coronary artery disease and hypertension. Myocardial fibrosis, hypertrophy, and cardiomyocyte apoptosis are typical features and can culminate in heart failure.

Both hUCMSC therapy and hUCMSC-derived exosomes have produced cardioprotective findings in experimental diabetes. Intravenous hUC-MSC administration in diabetic mice improved structural and functional signs of DCM and restored intramyocardial miRNA-133a expression while lowering systemic and local inflammatory mediators (Liu et al., 2024). MiR-133a targets COL1A1, a pro-fibrotic gene, and may therefore reduce collagen I deposition and myocardial fibrosis. MiR-100-5p carried by hUCMSCs-Exos targets FOXO3, inhibits NLRP3 inflammasome activation, and reduces IL-1β and IL-18 release, which can limit cardiomyocyte pyroptosis in diabetic conditions (Liang et al., 2020). MSC-derived exosomes have also reduced sterile inflammation and necroptosis through the TAK1-pJNK-NF-κB pathway, alongside improved cardiac function and less hypertrophy and fibrosis (Banerjee & Singla, 2024).

4.4. Diabetic Retinopathy

Diabetic retinopathy (DR) causes acquired visual loss in many working-age adults. Its progression involves retinal microvascular injury, breakdown of the blood–retinal barrier, and neuroretinal degeneration.

Evidence from experimental work suggests that hUCMSCs-Exos may slow pathological changes in DR. In a mouse model, miR-17-3p within hUCMSC-derived exosomes targeted STAT1 and reduced inflammatory and oxidative injury. Lower cytokine expression and preservation of retinal microvascular endothelial-cell integrity were reported (Li et al., 2021). Protection of retinal pericytes and endothelial cells from hyperglycaemia-associated apoptosis, together with suppression of pathological neovascularisation, may help preserve visual function.

5. Engineered Exosomes: Enhancing Therapeutic Potential

Natural hUCMSCs-Exos have limitations that are relevant to clinical translation. They do not necessarily reach target tissues efficiently, their cargo concentration may be inadequate, and the mononuclear phagocyte system can clear them rapidly. Engineering approaches seek to improve these properties.

5.1. Parent Cell Preconditioning

The parent hUCMSC can be modified before exosomes are collected. Hypoxia, pharmacological treatment, including autophagy-activating rapamycin, and selected signalling agonists can alter exosome biogenesis and increase the abundance of particular proteins and miRNAs (L. Wang, J. Chen, et al., 2024). In one example, small EVs from rapamycin-pretreated hUCMSCs improved diabetic wound healing by limiting macrophage-mediated inflammation and promoting angiogenesis through PI3K/AKT signalling (Zhang et al., 2025).

5.2. Direct Cargo Loading and Surface Modification

Direct cargo loading adds a selected therapeutic molecule to already isolated exosomes. Synthetic miRNA mimics, siRNAs, and small-molecule drugs can be introduced by electroporation, sonication, or freeze–thaw cycling. The resulting vesicles can protect cargo that would otherwise be degraded quickly or would not readily cross cell membranes. Exendin-4-loaded hUCMSC-derived exosomes prepared by electroporation have shown stronger renoprotective effects in DN models (L. Wang, A. Liang, et al., 2024).

Surface modification addresses the problem of tissue targeting. Targeting peptides, antibodies, and aptamers can be added to the exosome membrane, either by engineering the parent cell or by chemical conjugation after isolation. The intended result is a higher concentration at injured tissue and less exposure of non-target tissues (Li et al., 2021).

5.3. Biomaterial-Assisted Delivery Systems

Exosomes administered at a local site can leave that site quickly, which is a particular concern for diabetic wounds. For this reason, hUCMSCs-Exos are being combined with biomaterials. Injectable, self-healing, biocompatible hydrogels can protect the vesicles and provide sustained local release. In addition to maintaining bioactivity, this matrix can influence the wound environment in favour of regeneration (Zhang et al., 2025). Engineered exosomes within intrinsically immunomodulatory hydrogels have sequentially altered inflammation and promoted angiogenesis in diabetic wound models, suggesting a route to planned multi-phase treatment (Huang et al., 2025).

6. Challenges and Future Perspectives

Preclinical studies support further investigation of hUCMSCs-Exos, but routine clinical use will require several unresolved problems to be addressed.

A persistent difficulty is the absence of a common approach to exosome isolation and characterisation. Ultracentrifugation, size-exclusion chromatography, and precipitation methods do not produce identical yields or purity and may affect vesicle integrity differently. Without universally accepted Good Manufacturing Practice (GMP)-compliant procedures, batches and studies remain heterogeneous, complicating reproducibility and regulatory review (J. Chen et al., 2021). Agreement is needed on particle-size assessment by nanoparticle tracking analysis, protein-marker analysis by Western blot and flow cytometry, and functional-cargo profiling.

Scale-up is a second manufacturing problem. Clinical treatment will require quantities of exosomes that conventional cell culture cannot readily supply. Bioreactor and hollow-fibre systems are being developed to increase upstream production (Ahn et al., 2022). Purification after production must also deliver a product with the purity and consistency expected for clinical use.

Regulatory treatment of exosome-based products has not yet converged internationally. Different jurisdictions classify them differently, which complicates the design of clinical-development programmes. More explicit expectations for chemistry, manufacturing, and control (CMC), as well as pharmacokinetic and pharmacodynamic evaluation, would support translation (C. K. Wang et al., 2024).

The long-term distribution and safety of hUCMSCs-Exos are not yet fully defined. Preclinical studies generally report a favourable safety profile, but longer toxicology studies are still needed. These should include assessment of off-target effects and immunogenicity in diverse patient populations before wider use is considered.

Computational methods may have a role in exosome research as well. Artificial intelligence (AI) and machine learning could be used to select miRNA cargo combinations, model interactions between exosomes and recipient cells, and assist the design of engineered vesicles for particular indications. Linking such methods to biomaterials science and precision medicine may support more focused regenerative strategies in T2DM.

7. Conclusion

hUCMSC-derived exosomes are being investigated as a cell-free approach for T2DM and its complications. Their miRNA, protein, and lipid cargo can influence insulin resistance, β-cell injury, and repair processes in the kidney, heart, retina, and chronic wounds. Low immunogenicity, a favourable safety

profile, and movement across biological barriers are features that distinguish them from conventional drug treatments. The findings discussed in this chapter nevertheless remain largely preclinical.

Preconditioning of parent cells, direct cargo loading, surface functionalisation, and biomaterial-assisted delivery provide ways to refine hUCMSCs-Exos for specific uses. Clinical progress will depend on reproducible isolation and characterisation, scalable GMP production, a clearer regulatory framework, and adequately designed clinical trials. The value of hUCMSCs-Exos in diabetology will depend on how these requirements are met.

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CHAPTER 22

Challenges in the Prehospital Emergency Care of Older Adults: Clinical Complexity and System-Level Barriers

Songül Demir[1]

[1] Assistant Professor, Hatay Mustafa Kemal Üniversitesi Hatay Sağlık Hizmetleri Meslek Yüksekokulu, ORCID ID: 0000-0001-7170-429X

1. Introduction

Population ageing is increasing the demand for emergency medical services (EMS) among older adults. These patients frequently present with interacting clinical and contextual problems, including acute illness, frailty, multimorbidity, polypharmacy, cognitive impairment, functional decline, falls, and inadequate social support (Günay Tuzcu & Ekşi, 2025).

Traditional prehospital emergency medical services systems focus primarily on the rapid identification of life-threatening conditions, ensuring stabilization at the scene or during transport, and transporting patients to emergency departments (Al-Shaqsi, 2010). While the traditional prehospital emergency medical services model is vital for time-critical situations, it may fall short in meeting the complex care needs of older adults (Lederman et al., 2021). Prehospital emergency services use in later life is consequently shaped by multiple factors, including chronic conditions, medication burden, cognitive and psychological problems, living arrangements, previous healthcare use, and access to community services (Günay Tuzcu & Ekşi, 2025).

This chapter examines the principal clinical, ethical, and system-level challenges affecting older adults in prehospital emergency care. It argues that older adults require not simply more EMS care, but a geriatric-sensitive model integrating emergency treatment with frailty, cognition, function, medication burden, social context, person-centred decisions, and coordinated referral pathways.

2. Why Older Adults Require a Different Prehospital Approach

2.1 Age-related physiological change and reduced reserve

Age-related physiological changes reduce homeostatic reserves in multiple organ systems and make older adults more susceptible to acute stressors (Alshibani et al., 2021; Harthi et al., 2025). For this reason, older patients who appear stable under normal conditions can rapidly become decompensated during episodes of infection, trauma, dehydration, bleeding, or medication-related events (Aykut et al., 2026)

The structural and physiological changes that occur in the cardiovascular, respiratory, neurological, and musculoskeletal systems with aging reduce the physiological reserve of older adults against trauma and acute illnesses. In the cardiovascular system, ventricular hypertrophy, decreased diastolic filling, prolonged contraction time, and increased arterial stiffness may cause the classic signs of shock—such as hypotension and tachycardia—to be less pronounced. In the respiratory system, reduced lung reserve, a weakened response to hypoxia and hypercapnia, and inadequate airway clearance increase the risk of pneumonia and respiratory failure, particularly following chest trauma. Brain atrophy and age-related cerebrovascular changes can lead to the progression of intracranial

hemorrhage before distinct clinical signs appear, resulting in delayed diagnosis. Additionally, sarcopenia impairs balance and mobility, increasing the risk of falls, while osteoporosis predisposes individuals to severe fractures even from low-energy trauma. (Alshibani et al., 2021).

2.2 Atypical and non-specific presentation

Conventional adult vital signs alone are not reliable for ruling out serious illness in older patients; relying solely on vital signs remains risky due to age-related differences in response (İlhan et al., 2025). A large systematic review and meta-analysis has shown that older adults are less likely than younger adults to experience tachycardia and fever during an infection (Tupper et al., 2025). Systolic blood pressure that appears to be within the normal range may indicate relative hypotension in an older patient (De Simone et al., 2024). The absence of tachycardia may reflect the effect of medication or a blunted physiological response (Yeat et al., 2026).

Nonspecific symptoms often include weakness, fatigue, dizziness, confusion, recent falls, loss of appetite, gait disturbances, and functional decline. These symptoms do not provide sufficient information to follow a standard diagnostic pathway (Jackman et al., 2020). Because delirium, dementia, communication difficulties, multimorbidity, and polypharmacy make taking a history challenging, the underlying acute condition can easily be masked, and patients are sometimes unable to clearly explain why they were brought in. A clinically important point is that serious conditions such as pneumonia, myocardial infarction, or stroke may present with symptoms such as a fall, functional decline, confusion, or deterioration in general condition, rather than chest pain, fever, or focal symptoms (Shenvi & Platts-Mills, 2019). Nonspecific conditions can easily be overlooked in the highly stressful prehospital emergency medical services setting, and it has been observed that more patients fall into this category during periods of high workload. Nonspecific conditions can be associated with high morbidity and mortality. Between 51% and 59% of these patients have an acute, serious condition. (Kemp et al., 2020).

Older adults who present with atypical and nonspecific symptoms require a thorough evaluation and a meticulous approach; this involves gathering information from multiple sources. Understanding the patient within their biopsychosocial context and in light of their care goals enables personalized assessment, management, and referral (Jackman et al., 2020).

2.3 Multimorbidity and diagnostic uncertainty

In older patients, multimorbidity creates a clinical complexity that makes it difficult to recognize acute illness—not merely because of the increased number of diseases, but also due to clusters of interrelated conditions, functional decline, geriatric syndromes, and fragmented care (Alanazi et al., 2025; Forman et al.,

2018). This uncertainty can lead to “diagnostic masking,” in which new symptoms are mistakenly attributed to known diagnoses, and can result in a range of adverse outcomes, including inappropriate, costly, and potentially harmful diagnostic tests, as well as treatment conflicts in which the treatment of one disease exacerbates another (Alanazi et al., 2025). A study by Rosen et al. (2022) found that multimorbidity does not carry the same prognostic significance as having multiple comorbidities, and the details regarding this issue are important for determining the prognosis of individual and complex patients.

Although nonspecific presentations in older patients are common, they can easily be overlooked in emergency settings. These patients have an average of four comorbidities; their morbidity and mortality rates are high, and their list of discharge diagnoses is long and heterogeneous, which makes the differential diagnosis for these patients unusually broad.(Kemp et al., 2020). For this reason, prehospital clinicians should avoid attributing new symptoms in elderly patients solely to aging or known chronic conditions; because atypical presentations, multimorbidity, cognitive impairment, and polypharmacy can easily mask the presentation of acute illness and lead to new complaints being mistakenly attributed to existing diagnoses When diagnostic uncertainty persists, it is important to clearly state this during the handover; because transitions between prehospital emergency services and the emergency departments are critical moments for patient safety, and unstructured or incomplete communication can lead to harm through loss of information and uncertainty regarding responsibility (Alanazi et al., 2025; Dúason, Gunnarsson, & Svavarsdóttir, 2021).

2.4 Polypharmacy and medication-related risk

With the growing elderly population, the use of multiple medications is becoming increasingly common among older adults with multiple chronic conditions. Polypharmacy is associated with risks such as adverse drug events, hospitalizations, medication errors, drug-drug interactions, cognitive decline, and nonadherence to treatment. In older adults, cognitive decline, the complexity of treatment regimens, low health literacy, and fragmented care are among the factors contributing to medication nonadherence (Adeyemi et al., 2025).

Healthcare personnel working in the prehospital setting should evaluate acute clinical findings in elderly patients not only as a result of a primary pathology but also as a potential indicator of a medication-related adverse event (Budnitz et al., 2011). In addition to the prescription medications that an elderly patient takes regularly, systematically reviewing over-the-counter products, herbal products, and dietary supplements and conducting a medication reconciliation is a key component in determining the medication-related etiology of clinical presentations such as hypotension, hypoglycemia, syncope, or acute altered consciousness (Budnitz et al., 2011; Safari et al., 2022; Schepel et al., 2018).

In prehospital care, it is important to assess an elderly patient's medication history as thoroughly as possible in order to identify high-risk medication exposures—such as polypharmacy and the use of anticoagulants and antiplatelet agents—as well as pharmacological factors that could affect the acute clinical presentation (Harthi et al., 2022). Awareness of the potential inappropriate medication approach underlying the Beers or STOPP/START criteria; may facilitate the identification of potentially inappropriate medications, clinically significant drug-drug and drug-disease interactions, and pharmacological factors that could trigger or exacerbate acute clinical conditions such as falls, syncope, hypotension, hypoglycemia, bleeding, or altered consciousness (American Geriatrics Society, 2023; O'Mahony et al., 2023).

2.5 Functional, cognitive, and social context

Pre-hospital functional decline may be observed, particularly in older adults, in response to an acute medical illness. A better understanding of functional changes or decline in acutely ill older adults will create opportunities to consider early intervention and/or ways to modify outcomes. Older age, a low pre-hospitalization functional level (prior to the onset of the acute illness), the presence of dementia, and acute respiratory illnesses may be independently associated with pre-hospital functional decline (Palleschi et al., 2014).

Older adults may seek prehospital emergency medical services due to atypical symptoms and signs, such as inactivity and cognitive impairment. Although these atypical signs are part of geriatric syndromes, they can also be symptoms and signs of many acute illnesses. For this reason, healthcare professionals working in prehospital emergency medical services must conduct a thorough assessment (Hofman et al., 2017). Since frailty is an indicator of the risk of adverse outcomes, a decline in health or functional status among older adults may also serve as an indicator of frailty. There does not appear to be any risk screening tool routinely used in prehospital emergency medical services. A tool specific to prehospital emergency medical services (PERIL – Paramedics Evaluating Older Adults at Risk of Loss of Independence) is currently under development. Prehospital care providers can improve care for older adults by initiating the screening process and facilitating referral or transport to the most appropriate service. Paramedics are uniquely positioned to document an older adult's living conditions and functional status in their own home. A method is needed to capture this important information and compile it in a useful format (Goldstein, Andrew, & Travers, 2012).

Cognitive status is another critical parameter that directly influences prehospital assessment and decision-making processes. Prehospital emergency medical service providers frequently care for elderly patients with preexisting neurocognitive disorders (dementia) or acute brain dysfunction (delirium) (Kennedy et al., 2014; Voss et al., 2018). Healthcare professionals working in

prehospital emergency medical services report that, due to the lack of alternative services, they spend a great deal of time at the scene addressing the social needs of people with dementia. This situation may arise when healthcare professionals working in prehospital emergency medical services are unable to rule out other causes of cognitive impairment, such as sepsis or head trauma, or when they believe it is unsafe to leave the patient at home for social reasons (Voss et al., 2018). Since standard screening tests for delirium take at least 15 minutes, it will not be possible to formally screen all older adults for delirium in prehospital emergency medical services (Kennedy et al., 2014).

Dementia can pose a significant obstacle to clinical assessment in an emergency care setting; dementia-related confusion can lead to inaccuracies in the medical or medication history and may limit the individual's ability to understand questions or follow instructions. It is often difficult to determine whether confusion stems from a pre-existing cognitive impairment, delirium related to the incident that prompted the call, or a combination of both. In addition, paramedics must decide whether the patient can be safely treated and managed at home or in the community, or whether referral to a hospital is the most appropriate course of action. The preference to avoid unnecessary transports for patients with dementia, combined with the challenges of obtaining an accurate medical history and assessment, can make the decision-making process particularly challenging for paramedics (Voss et al., 2020).

Addressing the social context of older patients is not a secondary aspect of emergency care; it is an integral part of accurate risk stratification, making safe discharge decisions, and preventing unnecessary readmissions and emergency department visits (Berger et al., 2023). Paramedics, who spend one-on-one time with patients, are in a unique position to understand and address the social factors that contribute to patients' health. However, the assessment and management of social determinants have not been integrated into the practice of frontline paramedics. However, given that many emergency calls are non-urgent, exacerbated by social factors, and related to gaps in primary care services, all paramedics can play a role in addressing social determinants. As paramedics interact with patients, they can document important contextual and situational information about the living conditions that affect the patient's health (Allana & Pinto, 2021).

3. System-Level Barriers in the Prehospital Emergency Care of Older Adults

The clinical complexity associated with prehospital care for older adults is significantly exacerbated by a range of organizational, educational, and infrastructural barriers embedded in emergency medical services systems.

3. 1 Lack of Protocols and Clinical Guidelines Specific to the Elderly; The vast majority of prehospital emergency medical practices have been formalized

into protocols (Haussner et al., 2026). Although elderly trauma patients with one or more comorbid conditions may be at higher risk of death, clinical protocols and guidelines are also unclear on how to optimize the treatment and management of this population (Alshibani et al., 2021). Specific guidance practices for the care of older adults outlined in protocols have the potential to significantly improve the emergency care provided to older patients. However, there is limited guidance specific to geriatric care. Many guidelines for emergency services do not include any recommendations for assessing older adults in a wide range of emergency situations (Haussner et al., 2026).

3.2 Gaps in Education in Geriatric Emergency Medicine; Current prehospital training programs offer limited instruction in geriatric care, which leads to inadequate care (Shah et al., 2012). In a qualitative study conducted with ambulance staff, ambulance clinicians reported that they experienced knowledge gaps due to inadequate training in geriatric care during their university education and a lack of relevant training courses after they began working (Harthi et al., 2025).

Specialized training modules—such as the Geriatric Emergency Medicine Services Training program, which covers the assessment of elderly patients, environmental considerations, medication management, and social factors—can enhance knowledge and clinical practice in geriatric emergency care. These programs equip paramedics with the skills necessary to navigate the complexities of aging and provide holistic, evidence-based care (Alanazi et al., 2025).

3.3 Inadequate Triage and Lack of Triage Tools

Standard trauma triage criteria often lack sensitivity for older adults, which can lead to inadequate triage and inappropriate referral decisions (Alanazi et al., 2025). It is believed that an increase in inadequate triage among elderly trauma patients leads to higher mortality rates. Factors that may affect the accuracy of prehospital trauma triage criteria and triage decisions for older adults include altered physiological responses to trauma, injury mechanisms perceived as minor (such as falls from a low height, i.e., < 2 m), high rates of comorbidity and frailty, and polypharmacy (Alshibani , et al., 2021).

Understanding research priorities for older adult trauma patients—including prehospital care—is complex. Rapid and accurate prehospital triage and transport decisions are essential for older adult trauma patients. Studies indicate that more accurate geriatric-specific triage criteria need to be developed and that the integration of frailty assessment into the triage tool should be evaluated (Alshibani et al., 2021).

3.4 Decisions Not to Transport and Patient Safety Risks

A significant proportion of all patients who are not transported to the hospital after being assessed by ambulance personnel are older adults (Lederman et al.,

2021). Older adult patients often seek prehospital emergency medical services with stable vital signs. They may also seek care without presenting a specific chief complaint, such as changes in their general health, general weakness, feeling unwell, or an inability to perform daily activities. While these patients may be considered to not require critical care, they may still be experiencing serious health problems. (Farhat et al., 2023).

The decision-making process regarding non-transportation to a hospital requires ambulance professionals to possess adequate competencies, skills, and clinical judgment; However, their training curricula contains very little information on making transportation decisions. Furthermore, only a small number of ambulance services have developed non-transportation protocols and policies (Ebben et al., 2017).

3.5 Fragmented Care Transitions and Information Continuity

Older adults frequently transition between multiple care settings, and pre-hospital emergency medical services play a critical role in these transitions. The failure to transmit accurate and complete clinical information between these transitions, and the resulting gaps in documentation, are of great importance, particularly in older adult patients requiring emergency care. The frequent transitions of older individuals from long-term care to emergency departments and back again can lead to adverse events; effective communication between care settings is essential to ensure continuity of care, yet the rate of completion of standardized communication forms by emergency medical services during such transitions has been consistently low (McLane et al., 2022).

For paramedics, the consequences of information fragmentation are urgent and clinically significant. Without access to reliable drug lists, established diagnoses, developed advanced care plans, or baseline functional assessments, clinical decision-making in the pre-hospital setting is performed under conditions of significant uncertainty. Paramedics may also need information such as patients' end-of-life care requests and resuscitation status (Zorab, Robinson, & Endacott, 2015).

3.6 Workforce Constraints and Organizational Pressures

Emergency medical systems operate under significant time pressure, and on-scene time management is driven by operational goals and resource availability rather than solely clinical complexity. The comprehensive geriatric assessment required of older adult patients is a time-consuming, labor-intensive process involving gathering information from informal care partners or family members, and conflicts with the efficiency imperatives of emergency medical systems (Goldstein et al., 2014).

4. Conclusion

Older adults require not simply more prehospital emergency care but a different model of care. Acute illness and injury in later life occur within the context of reduced physiological reserve, frailty, multimorbidity, polypharmacy, cognitive and sensory impairment, functional limitations, and diverse social circumstances. These factors can alter clinical presentation, complicate assessment, influence treatment response, and increase the risks associated with both transportation and non-conveyance.

A geriatric-sensitive prehospital model should integrate immediate clinical severity with frailty, cognition, function, medications, social context, and individual goals of care. Structured assessment, age-sensitive clinical protocols, competent communication, appropriate pain management, supported decision-making, and integrated referral pathways are central to this transformation.

Emergency medical services should therefore evolve beyond a uniform assess–treat–transport model. Through geriatric education, clinical decision support, community paramedicine, interoperable information systems, and collaboration with primary, geriatric, social, and palliative care services, ambulance systems can deliver care that is safer, more individualized, and more responsive to what matters to older people. The central objective is not simply to determine where the patient should be transported, but to identify the most appropriate care pathway for that individual at that moment.

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CHAPTER 22

Digital Orthodontics: Contemporary Advances in Diagnosis, Treatment Planning, and Customized Appliance Fabrication

Abdeen Shaker[1]

[1] Assist. Prof. Dr., Department of Orthodontics, Faculty of Dentistry, Istanbul Atlas University, Istanbul, Türkiye, ORCID: 0000-0002-7855-5941

1. Introduction and Digital Transformation

Orthodontics is moving away from conventional impressions, plaster models and standardized prefabricated appliances toward integrated, patient-specific digital workflows (Panayi, Efstathiou, Christopoulou, Kotantoula, & Tsolakis, 2024; Zhang & Wu, 2026). Intraoral optical scanning (IOS), cone-beam computed tomography (CBCT) and 3D facial photogrammetry can now be combined into an integrated virtual patient for multidimensional diagnosis and treatment simulation (Panayi et al., 2024; Tarkan, 2025). The pace of technological adoption, however, has outstripped the evidence base. For the clinician, the relevant question is therefore no longer whether a digital tool exists, but what level of evidence supports its use.

Artificial intelligence (AI) has entered this framework as an automated diagnostic aid (Panayi et al., 2024). Deep learning performs well on narrowly defined image-processing tasks, such as automated 3D craniofacial superimposition for evaluating skeletal and dental change (Huang et al., 2026). Systematic review evidence, however, shows that AI treatment-planning systems remain constrained by variable training-data quality, risk of bias in primary studies and the absence of standardized prospective validation across clinical settings (Baxmann, Zsoldos, & Kárpáti, 2026). Technical precision on a test dataset and clinical validity are therefore not interchangeable.

Computer-aided design and computer-aided manufacturing (CAD/CAM) has expanded the range of patient-specific appliances (Panayi et al., 2024), but the evidence is more nuanced than is often assumed. In a laboratory comparison, as-fabricated selective laser melting (SLM) lingual brackets showed greater dimensional trueness error and slot friction than lost-wax cast brackets; polishing reduced the slot-height discrepancy to a non-significant level ($p = 0.088$), yet static friction remained significantly higher (1.59 ± 0.34 N vs. 1.25 ± 0.20 N; $p = 0.001$) (Nguyen, Trinh, Le, Nguyen, & Nguyen, 2025). For maxillary transverse deficiency, CBCT-guided customized miniscrew-assisted rapid palatal expansion (MARPE) appliances use 3D-printed metal frameworks adapted to the individual palatal vault (Mehta, Singh, Malhotra, Aziz, & Batra, 2025; Thakkar, Ghosh, & Keshwani, 2020; Thuy, Viet, Trang, Linh, & Anh, 2025; Viet et al., 2025). A separate line of clinical research shows that longer miniscrews achieving bicortical engagement produce greater basal bone expansion than monocortical engagement (Choi et al., 2023); this is a finding about anchorage, not about customization.

In clear aligner therapy, conventional thermoformed aligners effectively resolve mild-to-moderate crowding and manage non-extraction space closure (Baneshi, O'Malley, El-Angbawi, & Thiruvenkatachari, 2025; Muro, Caracciolo, Patel, Feres, & Roscoe, 2023). Predictability, however, is movement-specific. Uncontrolled buccolingual crown tipping and protocol-specific sagittal molar

distalization are relatively predictable (Haouili, Kravitz, Vaid, Ferguson, & Makki, 2020; Long & Park, 2026; Muro et al., 2023), whereas intrusion, extrusion, root torque and bodily translation remain difficult and frequently require overcorrection or auxiliary mechanics (Benedetti et al., 2025; Dai, Xu, & Shu, 2021; Haouili et al., 2020; Long & Park, 2026; Muro et al., 2023; Thilagalavanian, Weir, & Meade, 2025). Direct 3D printing with photocurable shape-memory resins eliminates the intermediate physical model and allows direct CAD control of appliance geometry, but it has not yet demonstrated clinical superiority over thermoformed systems (Kim, Miranda, & Kim, 2026; Torkomian, de la Iglesia, & Puigdollers, 2025; Zhang & Wu, 2026).

Digital retention relies on pre-debond intraoral scanning and virtual bracket removal (VBR) algorithms (ElShebiny, de Menezes, Stefanovic, Eliliwi, & Palomo, 2024; Xue et al., 2026). Prefabricated VBR retainers allow same-day delivery, showed no statistically significant six-month difference from conventional stone-model retainers, and reduced chairside time (Fu et al., 2025). Throughout this chapter, workflows already implemented clinically—intraoral surface acquisition, CAD/CAM indirect bonding trays, customized MARPE appliances, VBR retention and adjunctive AI diagnostic tools—are distinguished from emerging technologies such as direct 3D-printed aligners, whose evidence remains immature and whose routine adoption depends on further data on material viscoelasticity, in vivo monomer leaching and prospective clinical outcomes (Baxmann et al., 2026; Fu et al., 2025; Kim et al., 2026; Muro et al., 2023; Panayi et al., 2024; Viet et al., 2025; Zhang & Wu, 2026).

2. Digital Data Acquisition

Intraoral surface scanning is increasingly replacing conventional impressions and plaster models (Panayi et al., 2024). Scanners project structured light or laser onto dental and mucosal surfaces and stitch the captured frames algorithmically into a 3D surface, which is usually exported as a triangular STL mesh (Panayi et al., 2024); PLY and OBJ formats may additionally carry color or texture, depending on the export implementation. Five metrological concepts should not be conflated: trueness (agreement between the mean of repeated measurements and a reference value), precision (agreement among repeated scans), spatial resolution (the optical ability to resolve fine adjacent detail), mesh density (polygons per unit area—a property of the file, not of the optics) and clinical acceptability (the threshold below which error does not compromise diagnosis or appliance fit). A high mesh density, for example, does not by itself imply high trueness. Recognized sources of error include cumulative stitching drift during full-arch acquisition, light attenuation in interproximal areas, optical scatter from reflective restorations, salivary moisture and variation in the scanning path (Panayi et al., 2024).

CBCT provides volumetric hard-tissue information that optical surface scanning cannot (Panayi et al., 2024). A cone-shaped X-ray beam and a flat-panel detector produce isotropic voxel data exported in Digital Imaging and Communications in Medicine (DICOM) format, whose diagnostic value depends on field of view (FOV), voxel size, signal-to-noise ratio and metal or motion artifacts (Mehta et al., 2025; Panayi et al., 2024). Multi-planar reconstruction supports assessment of impacted teeth, alveolar bone height, apical root resorption, midpalatal suture maturation and temporomandibular joint morphology (Choi et al., 2023; Mehta et al., 2025; Panayi et al., 2024; Thuy et al., 2025). Because CBCT involves ionizing radiation, each examination must be justified individually. Exposure is governed by the As Low As Reasonably Achievable (ALARA) principle, which addresses overall dose minimization, and the As Low As Diagnostically Acceptable (ALADA) principle, which sets FOV, voxel size and exposure to the image quality the clinical question actually requires (Panayi et al., 2024). CBCT is therefore not a routine orthodontic record; it is reserved for complex situations—severe skeletal discrepancy, impacted teeth, surgical planning or CBCT-guided customized MARPE—in which the diagnostic benefit outweighs the radiation dose (Choi et al., 2023; Mehta et al., 2025; Panayi et al., 2024; Thuy et al., 2025).

Extraoral 3D facial scanning adds the soft-tissue envelope (Panayi et al., 2024). Besides stereophotogrammetry and desktop structured-light systems, smartphone scanning has attracted clinical interest (Panayi et al., 2024), but two technologies must be distinguished: active infrared dot-projection depth sensing (such as Apple's TrueDepth with the EM3D application) and passive structure-from-motion photogrammetry based on RGB photographs. Findings obtained with one cannot be generalized to the other or to all devices (Tarkan, 2025). In an in vivo comparison with direct caliper anthropometry, TrueDepth scans showed high test–retest correlations (Pearson r = 0.889–0.995 for patient self-scans and 0.847–0.995 for clinician scans) (Tarkan, 2025). Correlation coefficients, however, describe association rather than absolute agreement, and agreement was in fact region-dependent: mean differences were small for central lip measurements (stomion–labiale superius 0.25 ± 0.22 mm; stomion–labiale inferius 0.31 ± 0.29 mm) but exceeded 1 mm at peripheral landmarks (bigonial width 1.63 ± 1.13 mm; zygomatic width 1.02 ± 0.91 mm) (Tarkan, 2025). Clinician-acquired scans were faster and showed fewer motion artifacts than self-scans, whereas static scans cannot record dynamic soft tissue during speech or smiling, and facial hair, ambient light and peripheral distortion produce mesh defects (Tarkan, 2025). The method thus appears credible for the central face but less reliable for peripheral width measurements.

Intraoral scans, CBCT volumes and facial scans are complementary inputs for the integrated virtual patient (Panayi et al., 2024). Registered together, they support facially driven treatment planning, virtual articulator mounting (Jain &

Abduo, 2026) and virtual facebow orientation (Ellakany, Aggarwal, Khazaal, Kinderkenecht, & Ramtin-Zadeh, 2026).

3. The Integrated Virtual Patient

The integrated virtual patient is a multimodal 3D representation that combines dental surface geometry, skeletal and root anatomy and the facial soft-tissue envelope within a single coordinate system (Ellakany et al., 2026; Kobravi, Jafari, Lotfalizadeh, & Azimi, 2025; Lee & Kim, 2024; Panayi et al., 2024). Each modality contributes a distinct layer: intraoral scans (STL, PLY or OBJ) provide crown morphology and interocclusal relationships without radiation; CBCT (DICOM) supplies osseous architecture, root orientation and the relationships of impacted teeth; and facial scans record resting soft-tissue topography (Ellakany et al., 2026; Jain & Abduo, 2026; Kobravi et al., 2025; Panayi et al., 2024). A clear distinction must be drawn between a visually convincing 3D composite, assembled mainly for communication, and a genuinely registered dataset that can be used for diagnosis and planning (Ellakany et al., 2026; Jain & Abduo, 2026; Kobravi et al., 2025; Panayi et al., 2024).

Construction involves data acquisition, CBCT segmentation, surface reconstruction, initial alignment and registration refinement (Ellakany et al., 2026; Kobravi et al., 2025; Panayi et al., 2024). Segmentation may be threshold-based, manual, semi-automated or deep-learning-based, depending on tissue contrast and artifact severity (Kobravi et al., 2025; Panayi et al., 2024). Registration may be surface-based (e.g., Iterative Closest Point [ICP], which minimizes distances between overlapping meshes), voxel-based (aligning grayscale density distributions directly) or AI-assisted (neural networks predicting the spatial transformation) (Ellakany et al., 2026; Kobravi et al., 2025; Panayi et al., 2024). Accuracy should be reported separately for scanning, segmentation and registration (Ellakany et al., 2026; Jain & Abduo, 2026), because a highly accurate scan cannot compensate for a poor registration. Multi-planar inspection and color-coded surface distance maps, where available in the planning software, help verify alignment (Kobravi et al., 2025; Panayi et al., 2024).

Several challenges limit routine use (Kobravi et al., 2025; Lee & Kim, 2024; Panayi et al., 2024). Differences in posture and facial expression between facial scanning and CBCT acquisition produce non-rigid deformation, particularly in the perioral region (Ellakany et al., 2026; Panayi et al., 2024); streak artifacts from metallic restorations or fixed appliances obscure crown–root boundaries and complicate segmentation (Kobravi et al., 2025; Panayi et al., 2024); and incomplete surface overlap, landmark variability and proprietary file formats introduce further error (Jain & Abduo, 2026; Kobravi et al., 2025; Panayi et al., 2024). The evidence is additionally limited by heterogeneous registration protocols, scarce validation in severe craniofacial dysmorphology, unresolved

privacy issues concerning biometric facial data and a lack of prospective clinical validation (Baxmann et al., 2026; Jain & Abduo, 2026; Kobravi et al., 2025; Panayi et al., 2024).

When properly constructed, the virtual patient supports multidisciplinary applications (Kobravi et al., 2025; Lee & Kim, 2024; Panayi et al., 2024). In orthognathic surgery, virtual surgical planning (VSP) simulates multi-segment osteotomies, predicts 3D skeletal movements and allows the design of splints and guides (Anas, Sultan, & Orakzai, 2026; Kobravi et al., 2025; Lee & Kim, 2024); randomized trials and systematic reviews comparing it with manual model planning are discussed in Section 6 (Anas et al., 2026; Kobravi et al., 2025). Virtual facebows orient digital arches within virtual reference systems (Ellakany et al., 2026), and virtual articulators simulate static and dynamic occlusal contacts (Jain & Abduo, 2026). The virtual patient thus links multimodal diagnostic data to personalized CAD/CAM appliance design (Kobravi et al., 2025; Lee & Kim, 2024; Panayi et al., 2024; Tsolakis & Panayi, 2021).

4. Artificial Intelligence in Orthodontic Diagnosis

AI, machine learning (ML) and deep learning (DL) automate pattern recognition in 2D photographs, 3D surface scans and volumetric images (Baxmann et al., 2026; Panayi et al., 2024). Earlier decision-support systems were knowledge-based—rule-based expert systems, fuzzy logic and Bayesian networks in which reasoning rules were programmed by experts—whereas current systems rely mainly on deep neural networks that learn feature representations directly from clinical data (Baxmann et al., 2026; Panayi et al., 2024). Standalone software and online platforms now automate 2D cephalometric tracing, 3D CBCT segmentation and preliminary screening (Panayi et al., 2024). Their evaluation should follow four hierarchical levels: algorithmic accuracy (performance against reference labels on test data), clinical validity (accuracy against true anatomy across diverse populations), clinical utility (efficiency or decision-support benefit in practice) and treatment outcome improvement (demonstrably better long-term results) (Baxmann et al., 2026; Vaughan, Mheissen, Cobourne, & Ahmed, 2025). Strong performance at the first level does not imply benefit at the last.

Photograph-based screening illustrates the gap between availability and validity (Vaughan et al., 2025). A commercial platform evaluating intraoral smartphone photographs achieved an overall mean sensitivity of 72% and specificity of 54% across 19 dental and orthodontic parameters (Vaughan et al., 2025). Sensitivity was 100% for canine relationship, oral hygiene, gingivitis and recession, but only 44% for overjet and 13% for overbite; specificity was 9% for gingivitis and 6% for oral hygiene, producing frequent false positives, while canine relationship discrimination reached an area under the receiver operating characteristic curve (AUC) of 0.80 (Vaughan et al., 2025). Perfect sensitivity

combined with very low specificity is characteristic of a tool that flags almost every case, which limits its value as a diagnostic test. Overall agreement with direct clinical examination was only fair (Cohen's kappa = 0.29), a level that does not support autonomous malocclusion diagnosis without clinician supervision (Vaughan et al., 2025).

For 3D intraoral models, a two-stage Structure-Aware Long Short-Term Memory (SA-LSTM) network detected landmarks for curve of Spee (COS) assessment with a mean radial error of 0.32 ± 0.09 mm and a median COS depth error of 0.05 mm, requiring 0.02 s per model in test-set evaluation and 0.8 s in independent validation (Ma et al., 2026). For CBCT, deep learning automates segmentation and 3D craniofacial superimposition (Huang et al., 2026; Panayi et al., 2024). A multicenter validation of an open-source workflow combining skull segmentation (AMASSS) and registration (AReg) in growing Class II patients found mean absolute linear and angular differences generally below 1.5 mm and 1.5° relative to conventional voxel-based registration in cranial base, maxillary and mandibular superimpositions (Huang et al., 2026). These values express agreement with a reference method rather than clinical outcomes, and clinician verification remains necessary before interpretation (Huang et al., 2026).

For treatment planning, a systematic review of 19 studies found that several models showed high agreement with expert decisions, although performance varied across tasks, datasets and validation methods (Baxmann et al., 2026). Risk of bias was moderate to serious (ROBINS-I) because of retrospective designs, single-center convenience samples and the use of expert opinion as the reference standard (Baxmann et al., 2026). The last point is decisive: a model trained and tested against the decisions of clinicians from a limited number of institutions learns local decision patterns, and high agreement demonstrates reproduction of those patterns rather than validated correctness (Baxmann et al., 2026). Variable image quality, demographic bias, the "black-box" opacity of deep networks, data privacy and regulatory oversight impose further constraints (Baxmann et al., 2026; Panayi et al., 2024; Vaughan et al., 2025). AI should therefore be regarded as an adjunctive decision-support tool that improves efficiency and screening without replacing the orthodontist's comprehensive assessment or professional responsibility (Baxmann et al., 2026; Panayi et al., 2024; Vaughan et al., 2025).

5. Digital Treatment Planning and Predictability

Digital planning enables 3D visualization of the malocclusion, virtual setups, simulated staging and pre-treatment evaluation of goals (Arveda et al., 2025; Kim et al., 2026; Panayi et al., 2024). In an international modified Delphi study, all panel experts (100%) considered a digital setup necessary when planning clear aligner therapy (Arveda et al., 2025); consensus on necessity, however, is not evidence of predictability (Arveda et al., 2025). Four levels should be kept separate: virtual simulation (the displacement prescribed in CAD software),

biomechanical feasibility (what force systems and materials can physically deliver), actual tooth movement (the displacement achieved in the mouth) and final outcome (the aesthetic, functional and occlusal result after settling and retention) (Arveda et al., 2025; Daraze, Moussallem, Khoury, Kassis, & Ghoubril, 2025; Kim et al., 2026; Panayi et al., 2024). A virtual setup is an idealized geometric simulation that does not reproduce biological and biomechanical constraints and should never be read as a guaranteed outcome (Daraze et al., 2025; Kim et al., 2026; Panayi et al., 2024). Figure 1 illustrates these stages in one of the author's cases.



Figure 1. Digital treatment planning for a clinical case from the author's archive (the same case shown in Figures 2–4): (A) segmentation of roots and bone from CBCT data, (B) tooth segmentation in ArchForm, (C) virtual setup and staging of tooth movement in ClinCheck (Invisalign). Planning showed that the required expansion was skeletal and could not be achieved with clear aligners alone, which led to the use of MARPE. *Source: Author's clinical archive.*

Expression of a digital setup depends on malocclusion complexity, arch, movement type and anchorage management (Arveda et al., 2025; Daraze et al., 2025), and with clear aligners predictability differs markedly between movement categories (Benedetti et al., 2025; Dai et al., 2021; Haouili et al., 2020; Long & Park, 2026; Long & Park, 2026; Muro et al., 2023; Thilagalavanian et al., 2025). In extraction cases, setups help evaluate space closure options, incisor retraction and anchorage requirements (Daraze et al., 2025). A retrospective 3D study of maxillary first premolar extraction cases treated with fixed appliances (n = 62) showed that prediction accuracy depended on the anchorage protocol (Daraze et al., 2025). With reciprocal space closure (n = 22), predicted and achieved intercanine (p = 0.889), interpremolar (p = 0.212) and intermolar (p = 0.059) widths, as well as sagittal canine and molar positions ($p > 0.05$), did not differ significantly; with maximal anchorage (n = 40), significant discrepancies were found for intercanine ($p = 0.003$), interpremolar ($p < 0.001$) and intermolar ($p < 0.001$) widths and for sagittal molar position ($p < 0.05$) (Daraze et al., 2025). Two caveats apply. A non-significant difference in a small subgroup does not establish agreement, particularly when p = 0.059 lies close to the threshold; and the explanations proposed by the authors—anchorage loss, unplanned molar tipping or occlusal forces—are post-hoc hypotheses rather than measured variables (Daraze et al., 2025).

Biomechanics determine whether a CAD prescription becomes clinical reality (Arveda et al., 2025; Kim et al., 2026; Panayi et al., 2024). Delphi panelists agreed that unassisted aligners are of limited efficiency for complex vertical and bodily movements and recommended attachments or auxiliary mechanics (Arveda et al., 2025). With direct 3D-printed shape-memory aligners, malocclusion severity was reduced significantly, yet mid-course refinements were frequently needed for tracking loss or interarch adjustment (Knode et al., 2025). Moreover, reductions in the Peer Assessment Rating (PAR) index measure global improvement, not the accuracy with which individual planned movements were expressed (Knode et al., 2025).

AI is increasingly integrated into CAD software to generate preliminary setups (Baxmann et al., 2026; Panayi et al., 2024). As noted in Section 4, high agreement with experts on retrospective datasets from limited institutions does not establish generalizability to other clinical settings (Baxmann et al., 2026). Prediction accuracy should be assessed by comparing predicted and achieved tooth positions with validated 3D registration on stable references, such as palatal rugae superimposition in suitable maxillary cases (Daraze et al., 2025; Panayi et al., 2024). Responsibility for assessing biomechanical feasibility, monitoring tracking and performing refinements remains with the orthodontist (Arveda et al., 2025; Baxmann et al., 2026; Daraze et al., 2025; Kim et al., 2026; Knode et al., 2025; Panayi et al., 2024).

6. Digital Orthognathic Surgery

Orthognathic planning is moving from 2D cephalometry, analog facebow transfer and plaster model surgery to 3D VSP (Kobravi et al., 2025; Lee & Kim, 2024; Panayi et al., 2024). Conventional model surgery accumulates error from impression distortion, facebow registration and manual articulator positioning (Anas et al., 2026; Barakaat, Qabool, Tahir, Sukhia, & Fida, 2025; Kim, Donaldson, Wertheim, & Naini, 2024; Kobravi et al., 2025). VSP fuses CBCT, intraoral scans and facial photogrammetry into a virtual patient, allowing volumetric visualization of the deformity and interactive simulation of osteotomies (Anas et al., 2026; Kobravi et al., 2025; Lee & Kim, 2024; Panayi et al., 2024). It also provides a shared platform on which the orthodontist and surgeon can assess feasibility, coordinate presurgical decompensation, simulate soft-tissue change and refine objectives before surgery (Kobravi et al., 2025; Panayi et al., 2024).

The workflow follows a defined sequence (Kobravi et al., 2025; Lee & Kim, 2024; Panayi et al., 2024). After acquisition and registration, high-resolution optical dental scans replace the scatter-degraded CBCT dentition (Kobravi et al., 2025; Panayi et al., 2024). Le Fort I osteotomy, bilateral sagittal split ramus osteotomy (BSSO) or genioplasty is simulated through controlled translations and rotations of the segments relative to reference planes (Anas et al., 2026;

Kobravi et al., 2025; Lee & Kim, 2024). CAD software then generates intermediate and final wafers, cutting guides or patient-specific titanium plates (Barakaat et al., 2025; Kobravi et al., 2025; Lee & Kim, 2024; Lee et al., 2025), which are exported as STL files and milled or printed by stereolithography (SLA) or digital light processing (DLP) (Barakaat et al., 2025; Kim et al., 2024; Lee et al., 2025). Splints or guides transfer the plan to the surgical field before rigid fixation (Anas et al., 2026; Barakaat et al., 2025; Kobravi et al., 2025; Lee & Kim, 2024), and serial CBCT or surface superimposition is used postoperatively to compare the achieved and planned positions (Anas et al., 2026; Barakaat et al., 2025; Lee & Kim, 2024).

Accuracy must be evaluated at six levels: CAD design accuracy, manufacturing accuracy (device versus STL file), splint fit and seating, surgical transfer accuracy, postoperative skeletal positioning and patient-centered outcomes such as function, aesthetics and quality of life (Anas et al., 2026; Barakaat et al., 2025; Kim et al., 2024; Kobravi et al., 2025; Lee et al., 2025). Error can arise at each level, and excellence at one does not guarantee it at the next.

Systematic reviews indicate that VSP improves hard-tissue positioning accuracy compared with 2D planning (Anas et al., 2026; Barakaat et al., 2025; Lee & Kim, 2024), but the magnitude of the effect deserves scrutiny. A meta-analysis of 13 comparative studies (204 VSP and 213 conventional cases) found that VSP reduced the mean discrepancy between planned and achieved jaw positions by 0.54 mm (MD −0.54 mm, 95% CI −1.01 to −0.08, $p = 0.02$), with a larger difference in the custom surgical guide subgroup (MD −1.20 mm, 95% CI −1.87 to −0.54, $p = 0.0004$) (Lee & Kim, 2024). When the synthesis was restricted to 11 randomized controlled trials ($n = 240$), VSP produced only a modest improvement in maxillary vertical accuracy (MD −0.46 mm, 95% CI −0.92 to −0.01, $p = 0.04$, very low GRADE certainty), driven mainly by guide- and plate-based transfer (MD −0.89 mm) rather than splint-based transfer (MD −0.24 mm; subgroup difference $p = 0.001$), and maxillary horizontal accuracy did not differ (MD −0.41 mm, 95% CI −1.01 to 0.18, $p = 0.17$, very low certainty) (Anas et al., 2026). Operating time was shorter with VSP (SMD −2.53, 95% CI −4.45 to −0.60, $p = 0.01$, low certainty), whereas patient-reported quality of life and satisfaction did not differ (SMD 0.05, 95% CI −0.30 to 0.40, $p = 0.70$, low certainty) (Anas et al., 2026). Taken together, the gain is statistically significant but sub-millimetric and of low certainty; it appears to derive more from the transfer method than from virtual planning itself, and it has not yet translated into patient-reported benefit.

Manufacturing technology and resin selection influence transfer fidelity (Barakaat et al., 2025; Kim et al., 2024; Lee et al., 2025). In vitro, among 24 splints from 8 cases, subtractive milling (SM) achieved higher trueness (RMS

error 0.11 ± 0.02 mm) than DLP (0.14 ± 0.02 mm) or SLA printing (0.16 ± 0.02 mm; $p < 0.01$), with 88.89%, 70.53% and 61.22% of points, respectively, within a 0.1 mm tolerance ($p < 0.01$) (Lee et al., 2025). Paradoxically, DLP splints seated best on typodonts (8/8 vs. 6/8 for SM and 3/8 for SLA; overall $p = 0.028$; DLP vs. SLA $p = 0.01$), because enlarged intaglio perforations at incisal edges and cusp tips reduced seating interference (Lee et al., 2025). Trueness and fit are therefore different properties, and benchtop fit cannot be extrapolated to clinical superiority (Lee et al., 2025). Among SLA resins (90 splints), Surgical Guide resin was more accurate and precise than Dental LT V2 or Biomed Clear, yet Z-axis arch-length variation left only 33.3%–66.7% of splints within the manufacturer's ±0.1 mm tolerance (Kim et al., 2024). In clinical trials ($n = 203$), CAD/CAM splints showed only non-significant trends toward closer alignment in the Frankfort horizontal, coronal and sagittal planes (Barakaat et al., 2025), although one included trial reported no intraoperative splint modifications with CAD/CAM splints versus 58.3% with conventional splints ($p = 0.007$) (Barakaat et al., 2025). Error may originate from CBCT metal artifacts, segmentation and registration inaccuracy, polymerization shrinkage, Z-axis layer distortion and variable condylar seating (Anas et al., 2026; Kim et al., 2024; Kobravi et al., 2025; Lee et al., 2025; Panayi et al., 2024), while capital cost, learning curve, interoperability barriers, CBCT radiation and additional planning time call for careful case selection and clinical oversight (Anas et al., 2026; Barakaat et al., 2025; Kobravi et al., 2025).

7. CAD/CAM and 3D Printing

CAD/CAM has shifted appliance production from stone models and manual wire bending to digital design and automated fabrication (Panayi et al., 2024; Tsolakis & Panayi, 2021). The pipeline comprises five phases: data acquisition (IOS or CBCT), CAD design in dedicated orthodontic software (such as 3Shape Ortho System, OnyxCeph or Deltaface Ubrackets), export of open surface meshes (mainly STL or PLY), computer-controlled manufacturing, and post-processing with quality control (Palone et al., 2026; Panayi et al., 2024; Tsolakis & Panayi, 2021). In-office software allows clinicians to plan setups, customize bracket prescriptions and design appliances before production (Panayi et al., 2024; Tsolakis & Panayi, 2021).

Manufacturing is either subtractive or additive (Lee et al., 2025; Panayi et al., 2024). Five-axis CNC milling of pre-polymerized blocks (such as PMMA or hybrid ceramics) yields high surface trueness and dense material, but wastes material and is limited by cutter geometry and undercuts (Lee et al., 2025). Additive manufacturing builds objects layer by layer from the CAD file (Lee et al., 2025; Panayi et al., 2024) and includes vat photopolymerization (SLA, DLP and liquid crystal display [LCD]), fused deposition modeling (FDM) and powder bed fusion/SLM for metal alloys (Lee et al., 2025; Nguyen et al., 2025; Panayi et

al., 2024). SLM enables direct fabrication of cobalt–chromium (Co–Cr) lingual brackets and expansion frameworks, but as-fabricated surfaces require polishing to reduce slot friction, and polishing alone does not ensure clinical acceptability without rigorous quality control (Nguyen et al., 2025; Panayi et al., 2024).

Applications span diagnosis, active treatment and retention (Panayi et al., 2024). Printed resin models serve as casts for aligner thermoforming and VBR retainer prefabrication (ElShebiny et al., 2024; Torkomian et al., 2025; Xue et al., 2026). A slim, rigid CAD/CAM indirect bonding (IDB) tray tested clinically transferred virtually positioned brackets accurately while allowing visual confirmation of seating and removal of excess composite before light curing; the upper bounds of the group mean absolute errors were approximately 0.11 mm and 0.98°, but individual maximum errors reached 0.466 mm and 3.490° (Palone et al., 2026). Mean values can therefore mask clinically relevant outliers. CAD software also permits in-office printing of ceramic or resin brackets bonded with printed positioning keys (Panayi et al., 2024; Tsolakis & Panayi, 2021) and of metal-printed palatal expanders (Nguyen et al., 2025; Panayi et al., 2024).

Four parameters should be distinguished when evaluating CAD/CAM appliances: manufacturing accuracy (trueness of the object relative to the CAD reference), precision (repeatability across manufacturing cycles), clinical fit (intaglio adaptation and passive seating) and treatment effectiveness (performance in vivo) (Kim et al., 2024; Lee et al., 2025; Nguyen et al., 2025; Palone et al., 2026). As shown in Section 6, milling may achieve higher global trueness while DLP devices fit better on the bench because of relief perforations (Lee et al., 2025). Fidelity in additive systems depends on resin, layer thickness, light intensity, polymerization shrinkage, solvent washing and post-curing (Kim et al., 2024; Nguyen et al., 2025; Panayi et al., 2024). There is no universally optimal build orientation; it depends on the resin, printer, part geometry and application, and requires a balance between accuracy, build time and support placement (Kim et al., 2024; Lee et al., 2025; Panayi et al., 2024).

8. Customized Orthodontic Appliances and MARPE

Digital technology allows standardized hardware to be replaced by patient-specific components (Panayi et al., 2024; Tsolakis & Panayi, 2021), including CAD/CAM lingual and labial brackets with individualized bases and prescriptions (Hamiduddin, Aszkler, Warunek, Tanberg, & Al-Jewair, 2026; Nguyen et al., 2025; Tsolakis & Panayi, 2021) and digitally designed palatal expanders (Mehta et al., 2025; Thakkar et al., 2020; Thuy et al., 2025; Viet et al., 2025). Direct SLM metal printing or in-office hybrid-ceramic SLA printing allows brackets to be positioned according to the virtual setup; however, metal-printed components require polishing, and in the cited in vitro comparison friction remained significantly higher than in cast brackets despite polishing (Nguyen et al., 2025; Tsolakis & Panayi, 2021).

Customized MARPE is a digitally planned bone-borne or tooth-bone-borne treatment for maxillary transverse deficiency in late adolescents and adults (Mehta et al., 2025; Thakkar et al., 2020; Thuy et al., 2025; Viet et al., 2025). CBCT data are registered with intraoral scans (Mehta et al., 2025; Thakkar et al., 2020) to create a composite model in which miniscrews are positioned virtually in palatal safe zones (such as the anterior T-zone) to maximize cortical contact while avoiding the incisive canal, root apices and nasopalatine nerves (Mehta et al., 2025; Thakkar et al., 2020; Viet et al., 2025). The framework is designed to the individual palatal vault, addressing the poor adaptation of prefabricated expansion screws in narrow or high-arched palates (Thakkar et al., 2020; Viet et al., 2025). Fabrication may include direct Co–Cr printing, electropolishing, laser welding to the expansion mechanism and cementation before guided miniscrew insertion, although the steps vary across protocols and manufacturing systems (Mehta et al., 2025; Thakkar et al., 2020; Viet et al., 2025). Figure 2 shows these stages in the same case.

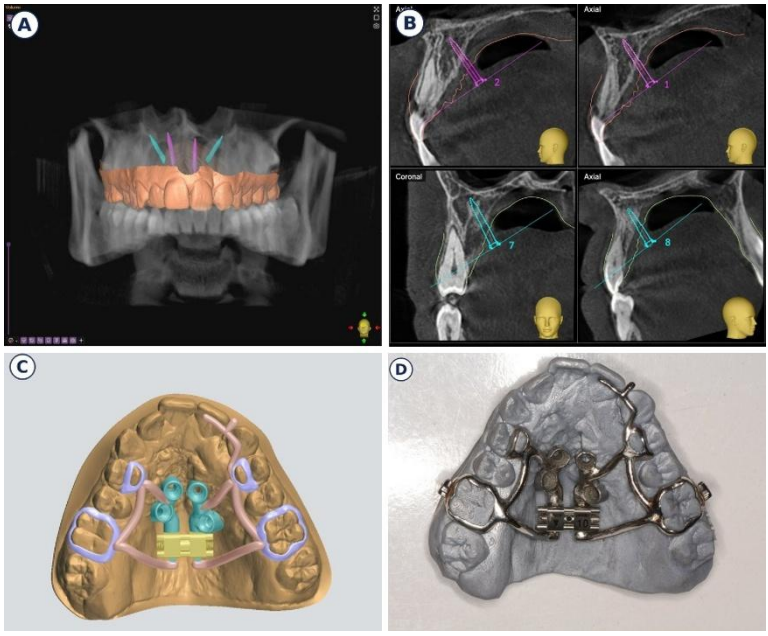


Figure 2. Design and fabrication of a customized MARPE appliance for the same case: (A) virtual miniscrew positioning on CBCT, (B) verification of miniscrew trajectories in axial and coronal sections, (C) CAD design of the customized framework, (D) the appliance after metal printing and post-processing, seated on the printed model.
Source: Author's clinical archive.

The number, trajectory, length and cortical engagement of miniscrews determine primary stability and force delivery (Choi et al., 2023; Mehta et al., 2025; Viet et al., 2025). In a randomized clinical trial (n = 31), long bicortical miniscrews (11–13 mm) engaging the palatal vault and nasal floor produced

greater basal bone expansion than short monocortical miniscrews (6–8 mm) (3.18 vs. 2.18 mm, $p = 0.010$), greater ectocanine expansion ($p = 0.001$) and better posterior miniscrew stability ($p = 0.024$) (Choi et al., 2023). Suture opening, however, did not differ significantly (83.9% overall; 75.0% long vs. 93.3% short, $p > 0.05$) (Choi et al., 2023); in a sample of this size, the numerically lower rate in the long-screw group is a reason for caution rather than a conclusion. More importantly, the trial compared miniscrew length, not customized versus conventional frameworks (Choi et al., 2023). The theoretical advantages of customization—better palatal adaptation and virtual placement planning—must be separated from demonstrated outcomes (Viet et al., 2025). Comparative evidence does not currently support universal superiority of customized MARPE over prefabricated appliances with respect to mucosal comfort, molar tipping or overall expansion success (Viet et al., 2025), and planning accuracy should not be equated with skeletal expansion, which is modulated by sutural resistance, skeletal maturity and the soft-tissue envelope (Choi et al., 2023; Thuy et al., 2025; Viet et al., 2025).

Customized MARPE does achieve transverse maxillary expansion (Mehta et al., 2025; Thuy et al., 2025; Viet et al., 2025). In a prospective CBCT study ($n = 15$), intercanine (+3.44 ± 2.57 mm), interpremolar (+3.72 ± 2.76 mm) and intermolar (+5.27 ± 2.18 mm) widths increased significantly at 6 months (all $p < 0.001$) (Mehta et al., 2025). These interdental widths, however, reflect combined skeletal and dental change rather than basal expansion alone, and non-significant changes in root resorption or alveolar height in 15 patients cannot be taken as proof that dental side effects are absent (Mehta et al., 2025). Philtrum height increased (+1.17 mm, $p = 0.019$) without significant change in alar width or airway volume (Mehta et al., 2025). A retrospective study ($n = 40$) using CBCT grey values reported a transient fall in suture density at 6 months, recovery by 12 months and radiographic suture continuity in all patients (Thuy et al., 2025); because grey values (Hounsfield Units) depend on scanner calibration and acquisition parameters, these represent relative radiographic changes rather than standardized bone mineral density (Thuy et al., 2025). Figure 3 shows the clinical response in the same case.

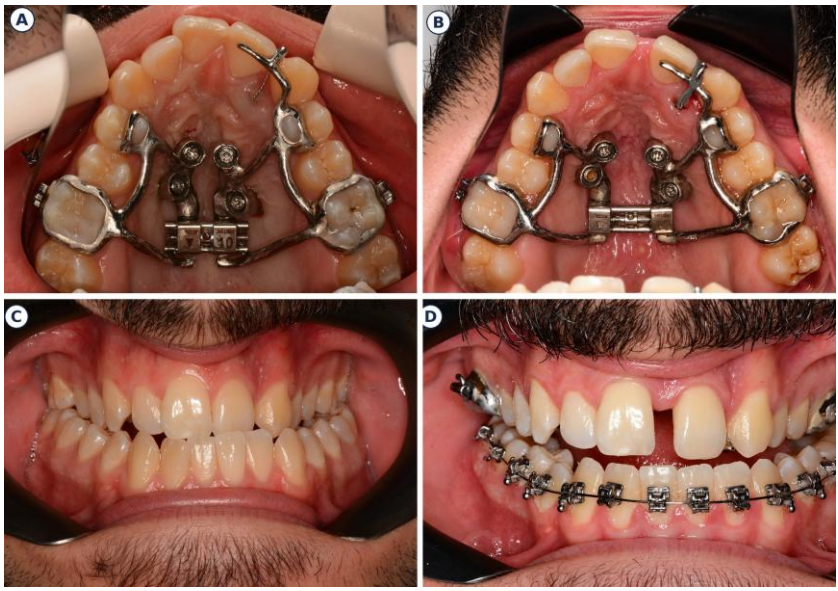


Figure 3. Delivery of the customized MARPE appliance and clinical response: (A) the appliance cemented intraorally, showing a traction arm integrated into the design for an impacted lateral incisor, (B) the appliance after activation, (C) before expansion, (D) after expansion, with a diastema between the maxillary central incisors as a clinical sign of midpalatal suture opening that should preferably be confirmed radiographically.
Source: Author's clinical archive.

Limitations include higher fabrication cost, software complexity and clinical risks such as miniscrew loosening, asymmetric expansion and transient mucosal inflammation (Mehta et al., 2025; Thuy et al., 2025; Viet et al., 2025). Evidence is lacking on post-retention stability beyond 12 months, on direct randomized comparison with conventional MARPE or surgically assisted rapid palatal expansion (SARPE), and on cost-effectiveness (Mehta et al., 2025; Thuy et al., 2025; Viet et al., 2025). Figure 4 shows the same case, in which the first appliance fractured after activation and had to be refabricated—a reminder that a digitally designed appliance remains subject to mechanical failure.



Figure 4. A metal-printed customized MARPE appliance that fractured after activation and was replaced with a new appliance in the same case.
Source: Author's clinical archive.

9. Clear Aligner Therapy: Clinical Effectiveness, Tooth Movement Predictability, and Direct 3D-Printed Aligners

9.1 Conventional Thermoformed Clear Aligners

Clear aligner therapy (CAT) has evolved from an aesthetic option for minor alignment into a modality applied to complex malocclusions (Baneshi et al., 2025; Muro et al., 2023; Panayi et al., 2024; Torkomian et al., 2025). Conventional aligners are produced by multi-stage thermoforming (Kim et al., 2026; Panayi et al., 2024; Torkomian et al., 2025): after intraoral scanning or impression, CAD staging generates a series of virtual setups (Arveda et al., 2025; Kim et al., 2026; Panayi et al., 2024); a model is printed for each stage, and sheets of single-layer polyethylene terephthalate glycol (PETG) or multilayer polyurethane/copolyester elastomers are vacuum- or pressure-formed over it, trimmed and polished (Kim et al., 2026; Panayi et al., 2024; Torkomian et al., 2025; Zhang & Wu, 2026).

Systematic reviews and meta-analyses comparing aligners with fixed appliances indicate that thermoformed aligners resolve mild-to-moderate crowding, manage non-extraction space closure and favor periodontal health during treatment (Baneshi et al., 2025; Muro et al., 2023). These favorable findings concern a narrower spectrum of cases than the range to which aligners are now applied. Thermoforming also has inherent limitations (Kim et al., 2026; Oyonarte et al., 2026; Torkomian et al., 2025; Zhang & Wu, 2026). Stretching heated sheets over resin casts produces non-uniform thinning—reaching 35% near occlusal and incisal line angles in some setups—and internal stress concentrations (Kim et al., 2026; Oyonarte et al., 2026; Torkomian et al., 2025). Errors accumulate across model printing, thermoforming and manual trimming, and conventional thermoplastics undergo rapid stress relaxation and force decay

at intraoral temperature and humidity (Kim et al., 2026; Oyonarte et al., 2026; Torkomian et al., 2025; Zhang & Wu, 2026).

9.2 Biomechanical Principles

Aligner force results from the elastic deflection of the polymer shell seated over a mismatched dentition (Long & Park, 2026; Oyonarte et al., 2026; Zhang & Wu, 2026). Its effective transmission depends on the anchorage–stress–contact triad: intimate contact between the intaglio surface and the crown, the stress generated within the material, and the resistance of anchorage units (Long & Park, 2026). Because smooth enamel offers little mechanical purchase, composite attachments of various geometries (rectangular, beveled or optimized) are bonded to create active pushing surfaces and directional force vectors (Arveda et al., 2025; Benedetti et al., 2025; Haouili et al., 2020; Long & Park, 2026).

To avoid unseating, setups cap stage velocity, typically at 0.20–0.25 mm of translation and 1.5°–2.0° of rotation per aligner, depending on the system and protocol (Arveda et al., 2025; Benedetti et al., 2025; Long & Park, 2026). Interproximal reduction (IPR) relieves contact tightness, creates space and prevents interproximal binding (Benedetti et al., 2025; Long & Park, 2026; Muro et al., 2023). When biomechanical demands exceed the capacity of the shell, Class II or Class III elastics, power arms, precision cuts and temporary anchorage devices (TADs) are used to reinforce anchorage and control force direction (Arveda et al., 2025; Dai et al., 2021; Long & Park, 2026; Muro et al., 2023). Figure 5 shows clinical examples.



Figure 5. Selecting the appropriate tool for the required movement: (A) clear aligners, (B) fixed appliances, (C) elastics supported by temporary anchorage devices (TADs).
Source: Author's clinical archive.

9.3 Tooth Movement Predictability

Global improvement of the malocclusion must be distinguished from the expression of individual planned movements (Haouili et al., 2020; Knode et al., 2025; Muro et al., 2023). Predictability varies with movement type, arch, root morphology and force delivery (Benedetti et al., 2025; Dai et al., 2021; Haouili et al., 2020; Muro et al., 2023; Thilagalavanian et al., 2025). Because studies quantify predictability with different methods and formulas, reported values must be interpreted within their study context rather than pooled directly (Benedetti et al., 2025; Haouili et al., 2020; Thilagalavanian et al., 2025). Figure 6 compares

the setup predicted in ClinCheck with the clinical result in a case treated with Invisalign.

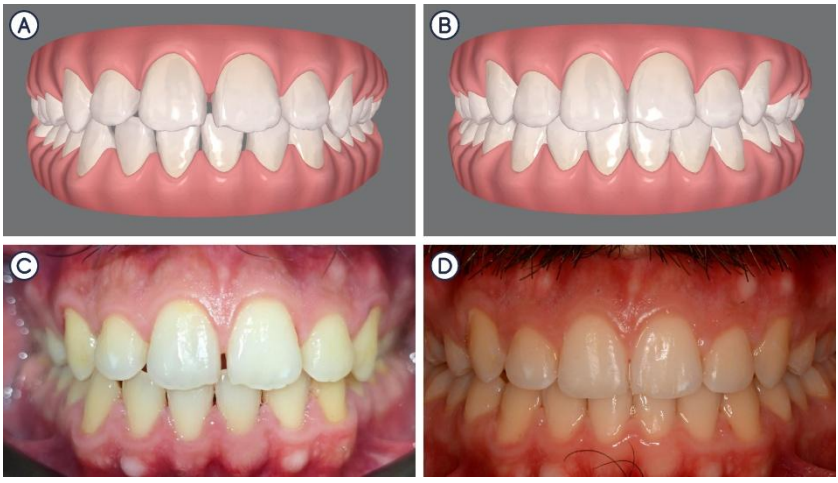


Figure 6. Predicted versus achieved outcome in a case treated with Invisalign clear aligners: (A) pretreatment virtual model and (B) final ClinCheck virtual setup showing the predicted tooth positions, (C) pretreatment frontal intraoral photograph, (D) posttreatment frontal intraoral photograph showing the achieved clinical result.

Source: Author's clinical archive.

9.3.1 Intrusion and Extrusion

Anterior vertical control remains a major challenge (Haouili et al., 2020; Long & Park, 2026; Muro et al., 2023). In a prospective 3D study of 38 patients, pure incisor intrusion reached a mean accuracy of only 33.4% for maxillary and 34.7% for mandibular central incisors, compared with 51.2% for maxillary second molar intrusion (Haouili et al., 2020). Posterior aligner lift-off and the lack of rigid anchorage have been proposed as the main reasons (Haouili et al., 2020; Long & Park, 2026). A modified Delphi panel recommended auxiliaries or miniscrews for unassisted bodily intrusion exceeding 1.50 mm in premolars, canines and maxillary molars, which reflects expert opinion rather than empirical proof of ineffectiveness (Arveda et al., 2025). Pure extrusion is similarly difficult because smooth crowns offer no active grip (Long & Park, 2026; Muro et al., 2023); unassisted incisor extrusion is the least accurate movement (18.3%–29.6%) and requires specific attachments or elastics (Muro et al., 2023).

9.3.2 Rotation

Rotational control depends on tooth morphology and location (Benedetti et al., 2025; Haouili et al., 2020; Muro et al., 2023). Flat-surfaced mandibular incisors reach up to 90% predictability, whereas canines and premolars with rounded crowns track poorly (Benedetti et al., 2025; Muro et al., 2023). Haouili et al. (2020) reported a mean rotational accuracy of 46.0%, with 28.0% for

mandibular first molar mesial rotation and 37.0% for maxillary canine distal rotation. A systematic review of 12 studies found a mean rotational accuracy of approximately 65% (range 36%–85%) with a mean absolute error of 2.3° (Benedetti et al., 2025); the width of this range itself shows how strongly results depend on system and protocol. Rotational increments below 1.5° per aligner are widely recommended to limit aligner deformation, although the reported benefit varies across protocols (Arveda et al., 2025; Benedetti et al., 2025).

9.3.3 Tipping, Torque, and Root Angulation

Uncontrolled crown tipping is the most predictable aligner movement because force acts directly on broad crown surfaces (Haouili et al., 2020; Muro et al., 2023). Overall buccolingual tipping accuracy was 56.0%, reaching 69.7% for labial crown tipping of maxillary lateral incisors (Haouili et al., 2020); that the most predictable movement achieves little more than half of the planned value is itself informative. Root torque control remains limited (Dai et al., 2021; Long & Park, 2026). In 17 adults treated with premolar extractions, maxillary central incisors tipped lingually rather than translating bodily, and the achieved change in inclination (−8.50°) far exceeded the planned change (−2.10°, $p < 0.001$) (Dai et al., 2021). Root angulation adjacent to extraction sites is also unpredictable: in 31 mandibular premolar extraction cases, expression ranged from −119.1% (movement opposite to the plan) to +162.8% (over-expression) (Thilagalavanian et al., 2025). For second premolar extractions, a 2-week wear protocol was associated with a 4.52° shortfall in root angulation compared with a 1-week protocol when crown tipping away from the extraction site was planned ($p = 0.005$); this is an association, not proof of causation. Optimized attachments offered no significant advantage over conventional attachments ($p > 0.05$) (Thilagalavanian et al., 2025).

9.3.4 Bodily Movement and Extraction Space Closure

True bodily translation during extraction space closure is a major obstacle for aligners (Baneshi et al., 2025; Dai et al., 2021; Long & Park, 2026). After first premolar extraction, aligners produced a “roller-coaster effect”, with anterior tipping and extrusion combined with posterior mesial tipping and anchorage loss: maxillary first molars lost 1.81 ± 1.19 mm more anchorage than predicted, and canine retraction fell 1.11 ± 0.85 mm short of the target (both $p < 0.001$) (Dai et al., 2021). Discrepancies of this magnitude are clinically relevant and explain the reliance on auxiliary anchorage described in Section 9.2.

9.3.5 Molar Distalization and Dental Arch Expansion

Narrative and scoping reviews describe sequential maxillary molar distalization as one of the more predictable sagittal movements, with efficacy of 80%–88% reported for 2.0–3.0 mm of distalization under 50% sequential staging (Long & Park, 2026; Muro et al., 2023). Distalization, however, occurs mainly

through distal crown tipping and causes flaring of the anterior anchorage teeth unless supported by Class II elastics or TADs (Long & Park, 2026; Muro et al., 2023). Delphi consensus recommends auxiliary anchorage for distalization exceeding 3.50 mm in the maxilla or 2.75 mm in the mandible, again reflecting expert opinion (Arveda et al., 2025). Aligners increase transverse interdental widths (Hamiduddin et al., 2026; Muro et al., 2023); in a cohort of 94 patients, maxillary intercanine expansion was closer to target with aligners than with custom 3D-printed brackets (mean target error 0.23 vs. 0.87 mm, $p = 0.013$) (Hamiduddin et al., 2026). Nonetheless, expansion with thermoformed aligners occurs predominantly through buccal crown tipping rather than basal bodily translation (Muro et al., 2023).

9.4 Direct 3D-Printed Aligners

Direct 3D-printed aligners (DPAs) are fabricated directly from CAD meshes without physical working models (Kim et al., 2026; Knode et al., 2025; Torkomian et al., 2025; Zhang & Wu, 2026). In a representative workflow, setups staged with velocity caps (e.g., 0.3 mm of translation or 4° of rotation per stage) are printed in photocurable resins such as Graphy Tera Harz TC-85 on SLA or DLP printers (Kim et al., 2026; Knode et al., 2025; Torkomian et al., 2025). Material-specific post-processing—centrifugal removal of unreacted resin, UV post-curing under nitrogen to optimize double-bond conversion, and thermal washing—is essential for structural integrity (Bleilöb, Welte-Jzyk, Knode, Ludwig, & Erbe, 2025; Kim et al., 2026; Knode et al., 2025; Torkomian et al., 2025). The approach eliminates model waste, reduces production steps and allows thickness to be varied by anatomical zone (Bleilöb et al., 2025; Kim et al., 2026; Torkomian et al., 2025; Zhang & Wu, 2026). Figure 7 compares the two fabrication workflows. Strictly speaking, however, direct printing does not simply shorten the workflow: although it eliminates the printing of working models, it adds two post-printing stages—resin cleaning, in which uncured resin is removed (e.g., by centrifugation), and light post-curing—both of which are critical to the final properties of the aligner.



Figure 7. Fabrication workflow of thermoformed aligners (TFAs) compared with direct-printed aligners (DPAs): the former involves printing a working model followed by thermoforming and trimming, whereas the latter is printed directly from resin, followed by removal of uncured resin and light post-curing.

Source: Prepared by the author.

In specific comparative setups, DPAs showed lower root mean square (RMS) deviation from the master STL than thermoformed aligners (0.140 mm vs. 0.188–0.209 mm) (Kim et al., 2026; Torkomian et al., 2025; Zhang & Wu, 2026), although fidelity depends on resin, printer, build orientation, post-curing and test conditions (Kim et al., 2026; Torkomian et al., 2025; Zhang & Wu, 2026). Micro-CT evaluation (Lim et al., 2026) showed larger initial passive gaps in TC-85 aligners (0.059–0.071 mm), attributed to polymerization shrinkage, but these gaps remained stable across activation levels ($p = 0.350$), whereas gaps in thermoformed aligners increased significantly with activation ($p < 0.001$) (Kim et al., 2026; Lim et al., 2026). Laboratory gap stability is not, however, proof of better clinical tracking (Kim et al., 2026; Lim et al., 2026). After 14 days of water immersion, printed resins softened viscoelastically and delivered much lower tensile forces (4.04–7.30 N for TC-85) than stiffer thermoplastics (26.26–65.23 N) (Oyonarte et al., 2026). Although TC-85 shows high shape recovery (86%–96%), water absorption (up to 8.7% over two weeks) and plasticization cause stress relaxation and force decay under static deflection (Kim et al., 2026; Oyonarte et al., 2026; Zhang & Wu, 2026). Whether these lower forces represent a biological advantage or a mechanical limitation for demanding movements remains an open question.

Clinically, a 12-month retrospective study of 54 patients with mild-to-moderate malocclusion treated with TC-85 DPAs reported a mean PAR reduction of 86.6% (from 17.01 ± 7.93 to 2.25 ± 1.15) (Knode et al., 2025), yet 20 of 49 active cases (40.8%) required mid-course refinement for IPR, interarch correction or incomplete tracking (Knode et al., 2025). Given the retrospective, uncontrolled design, the restriction to mild-to-moderate cases and the use of PAR, which

reflects global severity rather than movement accuracy (Kim et al., 2026; Knode et al., 2025), superiority over thermoformed aligners cannot be claimed in the absence of head-to-head randomized controlled trials (Kim et al., 2026; Knode et al., 2025; Torkomian et al., 2025; Zhang & Wu, 2026).

9.5 Clinical Limitations

Despite advances in software and materials, aligner therapy remains subject to biological and mechanical limits (Dai et al., 2021; Knode et al., 2025; Muro et al., 2023; Panayi et al., 2024). Virtual setups cannot account for periodontal ligament non-linearity, bone density, root morphology or patient compliance (Arveda et al., 2025; Long & Park, 2026; Panayi et al., 2024), and movement lag produces progressive tracking loss that necessitates refinements or fixed auxiliaries (Benedetti et al., 2025; Dai et al., 2021; Knode et al., 2025; Muro et al., 2023). In extraction cases and complex sagittal or vertical corrections, root torque, vertical control and anchorage are difficult to maintain without TADs or power arms (Arveda et al., 2025; Dai et al., 2021; Long & Park, 2026; Muro et al., 2023). The literature is methodologically heterogeneous, uses varying predictability formulas and relies largely on single-center retrospective designs and short-term laboratory models (Benedetti et al., 2025; Lim et al., 2026; Muro et al., 2023; Oyonarte et al., 2026). Long-term randomized controlled trials are needed to standardize staging protocols, compare direct-printed with thermoformed aligners and assess three-dimensional movement expression across diverse populations (Baneshi et al., 2025; Benedetti et al., 2025; Knode et al., 2025; Muro et al., 2023; Torkomian et al., 2025).

10. Virtual Debonding and Digital Retention

Conventional retention requires post-debond impressions or scans, model fabrication and laboratory thermoforming, which entails chairside waiting or a second appointment (Fu et al., 2025; Panayi et al., 2024). VBR digitally removes appliances from a pre-debond intraoral scan, shortening the interval between debonding and retainer delivery (ElShebiny et al., 2024; Fu et al., 2025; Panayi et al., 2024; Xue et al., 2026). The arch is scanned with brackets in place, the brackets are segmented and removed, the buccal enamel is reconstructed or replaced, a resin model is printed, and the retainer is thermoformed for delivery at debonding (ElShebiny et al., 2024; Fu et al., 2025; Panayi et al., 2024; Xue et al., 2026). VBR-based thermoformed retainers should be distinguished from directly printed retainers, which remain an emerging technology requiring clinical investigation (Fu et al., 2025; Panayi et al., 2024; Torkomian et al., 2025; Xue et al., 2026).

Enamel restoration after digital bracket removal uses either algorithmic surface reconstruction (RC) or anatomical surface replacement (RP) (Xue et al., 2026). RC fills mesh voids by curvature interpolation (ElShebiny et al., 2024; Fu et al., 2025; Xue et al., 2026), whereas RP registers pre-treatment crown

morphology onto the post-treatment scan (Xue et al., 2026). In vitro (14 paired models, 392 teeth), both workflows placed more than 96% of test points within a laboratory-defined 0.15 mm threshold, a criterion that does not in itself establish clinical fit (Xue et al., 2026). RC was more consistent, with lower RMS deviation on printed models (median 0.058 vs. 0.069 mm) and better internal fit of the retainers (median RMS 0.15 vs. 0.21 mm; both $p < 0.001$) (Xue et al., 2026). AI is now used to automate bracket identification and mesh repair (ElShebiny et al., 2024). In 30 arches from 17 patients, single-touch software removed brackets without manual editing under the study protocol, a result that cannot be assumed for all bracket types or for severe crowding (ElShebiny et al., 2024). Retainers made from post-debond reference scans scored higher for facial adaptation (2.00 vs. 1.56) and snap-fit (1.93 vs. 1.53; $p < 0.001$) than AI-derived retainers, although both were graded clinically acceptable (ElShebiny et al., 2024).

In a randomized clinical trial (38 enrolled, 36 completed), VBR retainers and conventional stone-model retainers did not differ significantly at six months in surface deviation (maxilla 0.18 ± 0.06 vs. 0.17 ± 0.06 mm; mandible 0.18 ± 0.05 vs. 0.19 ± 0.05 mm; $p > 0.05$) or arch form (Fu et al., 2025). In a trial of this size, the absence of a significant difference does not demonstrate equivalence or non-inferiority (Fu et al., 2025). VBR reduced visit duration at debonding (7.91 ± 1.19 vs. 64.98 ± 4.85 min, $p < 0.05$) within that trial's specific workflow, which may not be reproducible in all settings (Fu et al., 2025). Moreover, 10.5% of VBR retainers (2/19) required immediate refabrication because of inadequate posterior fit (Fu et al., 2025), so clinical inspection and adjustment remain necessary. Gaps in evidence include stability beyond six months, application to aligner attachments and lingual appliances, and standardized guidelines for the digital removal periphery (ElShebiny et al., 2024; Fu et al., 2025; Xue et al., 2026).

11. Biocompatibility and Safety

The biological safety of aligner materials involves polymer degradation, monomer leaching, microplastic release and host tissue response (Carganico et al., 2026; Kim et al., 2026; Panayi et al., 2024; Selvaraj et al., 2026; Zhang & Wu, 2026). Thermoformed aligners are made from chemically stable, pre-polymerized thermoplastics (such as PETG or polyurethane) with generally favorable cytocompatibility in vitro (Carganico et al., 2026; Kim et al., 2026; Zhang & Wu, 2026). DPAs, by contrast, are made from reactive photocurable resins—polyurethanes, methacrylates and aliphatic vinyl ester-urethane blends—whose cross-linked network depends on photopolymerization during printing and on material-specific post-processing (Carganico et al., 2026; Kim et al., 2026; Zhang & Wu, 2026). Their evaluation therefore focuses on residual monomers, photoinitiators, degradation products and microplastic particles (Carganico et al., 2026; Kim et al., 2026; Selvaraj et al., 2026; Zhang & Wu, 2026).

Leaching of urethane dimethacrylate (UDMA) is a primary concern (Kim et al., 2026; Selvaraj et al., 2026; Zhang & Wu, 2026). In 15 young adults wearing TC-85 aligners, stimulated salivary UDMA measured by high-performance liquid chromatography (HPLC) was below the detection limit (0.25 μg/L) at baseline—reported as 0.00 μg/L, which is not necessarily an absolute zero—and rose progressively to 4.04 ± 0.40 μg/L at 24 h ($p = 0.002$), 5.49 ± 0.46 μg/L at 7 days ($p = 0.002$) and 7.27 ± 0.46 μg/L at 14 days ($p = 0.001$) (Selvaraj et al., 2026). Reported in vitro median cytotoxic concentrations (TC_{50}) for UDMA (0.05–2.0 mM) correspond to approximately 23.53–941.12 mg/L (molecular weight 470.56 g/mol) (Selvaraj et al., 2026), so the 14-day peak lies roughly three orders of magnitude below the lower bound. Reassuring as this margin is, cell-culture lethal thresholds are not human safety limits (Selvaraj et al., 2026; Zhang & Wu, 2026). Chemical detection, in vitro cytotoxicity and clinical harm are distinct questions, and biweekly aligner replacement creates a recurrent exposure throughout treatment (Selvaraj et al., 2026; Zhang & Wu, 2026).

Cytocompatibility depends on formulation, physical state and exposure conditions (Bleilöb et al., 2025; Carganico et al., 2026). When abrasion-generated microplastics from eight aligner materials were applied to human dental pulp stem cells (hDPSCs), thermoplastics and polyamide-12 generally maintained viability above the 70% threshold of ISO 10993-5, whereas some printed photopolymers, including TC-85, reduced viability in a dose- and time-dependent manner, approaching moderate cytotoxicity at high particle concentrations (1000–5000 ng/100 μL) and prolonged exposure (72–96 h) (Carganico et al., 2026). With solid Tera Harz TA-28 discs (0.5–6.0 mm) incubated with human gingival fibroblasts (HGFs) after 20 min of nitrogen-atmosphere UV post-curing, viability decreased modestly with thickness ($p < 0.01$ for 2–6 mm) but remained above 70% at all thicknesses, and extending post-curing to 30 or 60 min conferred no additional benefit (Bleilöb et al., 2025). These TA-28 findings are specific to that formulation and protocol and cannot be extrapolated to TC-85 or other resins (Bleilöb et al., 2025; Carganico et al., 2026).

Post-processing parameters and the final degree of double-bond conversion (e.g., approximately 83% for TC-85 under specific nitrogen curing workflows) are material- and equipment-dependent rather than universal (Bleilöb et al., 2025; Kim et al., 2026; Selvaraj et al., 2026; Zhang & Wu, 2026). Nor can post-processing prevent intraoral degradation: water absorption (up to 8.7% over 14 days), thermal fluctuation, salivary enzymes and masticatory wear cause plasticization, surface roughening and continued leaching (Kim et al., 2026; Oyonarte et al., 2026; Selvaraj et al., 2026; Zhang & Wu, 2026). Current safety evidence rests largely on short-term static cell-culture models, with limited long-term human exposure data and no standardized clinical microplastic assays; prospective longitudinal clinical safety studies are therefore a priority (Carganico et al., 2026; Kim et al., 2026; Selvaraj et al., 2026; Zhang & Wu, 2026).

12. Clinical Implementation and Limitations

Implementing digital workflows requires investment in infrastructure, team training, quality control and professional oversight (Kim et al., 2026; Panayi et al., 2024; Zhang & Wu, 2026). An in-office laboratory can produce diagnostic models, IDB trays, occlusal splints, customized brackets and aligners (Panayi et al., 2024; Tsolakis & Panayi, 2021), but it entails substantial capital and running costs, and resin processing requires ventilated areas, separate solvent washing stations and handling protocols to limit volatile organic compound exposure and contact allergy among staff (Kim et al., 2026; Panayi et al., 2024; Zhang & Wu, 2026).

Interoperability remains a central challenge (Baxmann et al., 2026; Kim et al., 2026; Panayi et al., 2024): differences in file formats (STL, PLY, DICOM), mesh density or coordinate systems between scanners, CBCT units, CAD software and manufacturing hardware can introduce registration drift or dimensional distortion before fabrication (Kim et al., 2026; Panayi et al., 2024). Quality assurance—verification of printed appliances against CAD specifications and routine calibration of layer thickness, light intensity and resin tank clarity—is therefore required at every stage (Kim et al., 2026; Panayi et al., 2024; Zhang & Wu, 2026). Even a precisely manufactured IDB tray requires intraoral seating verification and removal of excess adhesive before light curing (Palone et al., 2026); manufacturing precision does not replace clinical execution.

Digital setups and AI tools are decision-support aids that do not remove the limits of clinical prediction (Arveda et al., 2025; Baxmann et al., 2026; Panayi et al., 2024); because current planning models are trained largely on retrospective, single-center clinician choices, all proposals require clinician review and verification (Baxmann et al., 2026; Panayi et al., 2024). Nor does digitalization uniformly reduce cost or chairside time: VBR can shorten debonding visits in optimized settings (Fu et al., 2025), but CAD design, model editing and printing troubleshooting demand considerable staff and clinician time (Panayi et al., 2024). Digital records and cloud-based platforms raise further issues of privacy, secure storage and informed consent for third-party processing (Baxmann et al., 2026; Kobravi et al., 2025; Panayi et al., 2024). Technology supports, but does not supersede, the orthodontist's judgment and professional responsibility (Arveda et al., 2025; Baxmann et al., 2026; Kim et al., 2026; Panayi et al., 2024).

13. Future Perspectives

Intraoral scanning, printed diagnostic models, automated landmark identification, CAD/CAM indirect bonding and VBR are established in clinical practice (ElShebiny et al., 2024; Fu et al., 2025; Huang et al., 2026; Palone et al., 2026; Panayi et al., 2024; Vaughan et al., 2025; Xue et al., 2026), whereas emerging applications require rigorous prospective validation before routine use (Baxmann et al., 2026; Kim et al., 2026; Torkomian et al., 2025; Zhang & Wu,

2026). Research priorities include validation of AI planning platforms on multicenter prospective cohorts against long-term outcomes rather than retrospective clinician decisions (Baxmann et al., 2026); virtual patients capable of dynamic occlusal and soft-tissue simulation with minimal multimodal registration drift (Ellakany et al., 2026; Jain & Abduo, 2026; Panayi et al., 2024; Tarkan, 2025); and evidence on whether customized MARPE produces stable skeletal or airway changes beyond 12 months, together with optimized bicortical and density-based miniscrew placement (Choi et al., 2023; Mehta et al., 2025; Thakkar et al., 2020; Thuy et al., 2025; Viet et al., 2025).

For direct-printed aligners, laboratory gap stability (Lim et al., 2026) and viscoelastic behavior (Oyonarte et al., 2026) must be translated into demonstrated clinical predictability through randomized trials against thermoformed aligners (Baneshi et al., 2025; Kim et al., 2026; Knode et al., 2025; Muro et al., 2023; Torkomian et al., 2025; Zhang & Wu, 2026), and material research should address long-term in vivo UDMA elution (Selvaraj et al., 2026), intraoral microplastic degradation and cytocompatibility under wear and masticatory fatigue (Bleilöb et al., 2025; Carganico et al., 2026; Selvaraj et al., 2026; Zhang & Wu, 2026). Multi-year trials of retention stability after VBR are needed (Fu et al., 2025), and, building on automated superimposition already validated in growing patients (Huang et al., 2026), serial superimposition and remote monitoring for early detection of tracking loss and relapse still require clinical validation (Muro et al., 2023; Panayi et al., 2024). All of these developments depend on open interoperability, standardized quality control in additive manufacturing and robust data protection (Baxmann et al., 2026; Kim et al., 2026; Kobravi et al., 2025; Panayi et al., 2024).

14. Conclusions

Digital orthodontics is evolving into integrated workflows spanning diagnosis, virtual planning, personalized appliance fabrication and retention (Fu et al., 2025; Kim et al., 2026; Panayi et al., 2024), with genuine gains in visualization, customization and efficiency (Baxmann et al., 2026; Huang et al., 2026; Lee & Kim, 2024; Mehta et al., 2025; Panayi et al., 2024; Thakkar et al., 2020; Thuy et al., 2025; Torkomian et al., 2025; Vaughan et al., 2025). The evidence, however, is uneven: well-supported diagnostic tools coexist with promising modalities, such as direct-printed aligners, that have not demonstrated clinical superiority over conventional alternatives (Anas et al., 2026; Baneshi et al., 2025; Baxmann et al., 2026; Kim et al., 2026; Knode et al., 2025; Muro et al., 2023; Torkomian et al., 2025; Viet et al., 2025; Zhang & Wu, 2026). Where statistically significant benefits have been shown, as with VSP, they are often small and of low certainty, and should be weighed accordingly.

Simulation fidelity and sub-millimeter manufacturing precision do not guarantee biological predictability, adequate force expression or clinical success

(Arveda et al., 2025; Daraze et al., 2025; Kim et al., 2026; Lee et al., 2025; Lim et al., 2026; Oyonarte et al., 2026; Panayi et al., 2024). Aligner movements differ markedly in predictability, with uncontrolled tipping and protocol-specific distalization more predictable than vertical control, root torque or bodily translation (Benedetti et al., 2025; Dai et al., 2021; Haouili et al., 2020; Long & Park, 2026; Long & Park, 2026; Muro et al., 2023; Thilagalavanian et al., 2025); virtual setups cannot model biological response, sutural resistance or compliance (Arveda et al., 2025; Choi et al., 2023; Daraze et al., 2025; Long & Park, 2026; Panayi et al., 2024); and material biocompatibility, quality control, standardized post-processing and long-term stability remain essential to patient safety (Bleilöb et al., 2025; Carganico et al., 2026; Fu et al., 2025; Selvaraj et al., 2026; Xue et al., 2026; Zhang & Wu, 2026). AI and automated CAD proposals reflect retrospective training data and developer preferences rather than validated standards of care (Baxmann et al., 2026). Technology is therefore an adjunct, not an autonomous decision-maker: the orthodontist retains responsibility for diagnosis, critical appraisal of digital setups, verification of appliance fit and ongoing monitoring, guided by biomechanical principles, sound evidence and clinical judgment (Arveda et al., 2025; Baxmann et al., 2026; Kim et al., 2026; Panayi et al., 2024).

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CHAPTER 22

Sources of Parasitic Infection Risks Among Home-Care Patients: Dynamics of Transmission and Strategies for Prevention

Muhammed Yasul[1]

[1] Öğr. Gör. Dr., Program of Home Healthcare, Department of Healthcare Services, Vocational School of Health Services, Bartın University, Bartın, Türkiye.
ORCİD: 0000-0001-7948-5773

Introduction and Epidemiological Framework: Strategic Significance of Parasitic Infections in Home-Care Patients

Global demographic aging has led to an ever-growing demand for healthcare services worldwide. Driven by advancements in medical technology and increased life expectancy, this demographic shift continuously expands the chronic patient population, thereby elevating the need for both professional institutional and home-care interventions. Consequently, home-based healthcare services have expanded rapidly, aiming to alleviate inpatient bed capacity pressures, curb medical expenditures, and enable patients to maintain independence and comfort within their domestic environments. Nevertheless, while this transition mitigates exposure to nosocomial infections, it simultaneously introduces distinct biological hazards native to the home setting. Immunocompromised and bedridden individuals face a significantly heightened vulnerability to parasitic pathogens. Identifying specific risk factors driving parasitic transmission in home care and formulating targeted preventive strategies are therefore imperative for optimizing clinical success and informing robust public health policies (Zeynudin et al., 2013; Shang et al., 2018; Çayır, 2020; ACSQHC, 2021; WHO, 2021; TÜİK, 2024).

1. Epidemiological and Clinical Profile of the Home-Care Population

Epidemiological profiling indicates that home healthcare recipients constitute a highly vulnerable demographic characterized by heightened susceptibility to parasitic pathogens and opportunistic infectious agents. Demographically, this cohort is overwhelmingly composed of geriatric individuals, with a statistically significant female predominance. Although transition to home-based care is primarily driven by the intent to minimize hospitalization duration, reduce healthcare costs, circumvent nosocomial transmission risks, and preserve patient autonomy within domestic settings, residential environments frequently suffer from suboptimal infection control measures and lax hygiene protocols. This environmental deficit is further compounded by the widespread omission of preventive healthcare strategies, most notably routine influenza and pneumococcal vaccination. Such immunologic gaps diminish systemic host defenses, thereby predisposing chronic and elderly patients to severe secondary manifestations of opportunistic parasitic infections. Furthermore, the requirement for long-term invasive clinical procedures particularly indwelling urinary catheterization disrupts mucosal barrier integrity and facilitates microbial colonization. Conclusively, the synergistic interplay between invasive interventions, insufficient immunization, and compromised domestic hygiene drives severe disease progression and elevates morbidity in this immunocompromised population (Zeynudin et al., 2013; Shang et al., 2018; Yılmaz et al., 2018; Çayır, 2020; TÜİK, 2024).

1.2. Analysis of the Domestic Setting: Infection Risk Factors and Environmental Dynamics

Unlike controlled hospital environments, domestic settings lack standardized sterilization and rigorous infection-control protocols. Environmental factors including inadequate ventilation, elevated ambient humidity, communal living spaces, and deficient sanitation exert a direct influence on the environmental persistence, surveillance, and host transmission dynamics of parasitic agents. Indeed, the improper disposal of household waste and contaminated medical materials, combined with the presence of domestic pets and frequent exposure to disease vectors, enhances the environmental resistance and viability of parasitic eggs and larvae. This scenario establishes a substantial infection risk for both cohabiting family members and healthcare providers. When compounded by a patient's physiological frailty and cognitive impairment, these environmental and epidemiological hazards create a susceptible biological and behavioral milieu that facilitates host colonization by parasitic pathogens (Robertson et al., 2000; Shang et al., 2018; Adams et al., 2021).

2. Pathophysiological and Behavioral Analysis of Host-Related Risk Factors

Chronic pathologies and functional incapacities observed among home-care patients must be recognized not merely as isolated clinical diagnoses, but as biological and behavioral pathophysiological drivers that directly instigate parasitic transmission, tissue invasion, and autoinfection cycles (Zeynudin et al., 2013; Luvira et al., 2022).

2.1. Immobilization, Bedridden Status, and Disruption of Skin Barrier Integrity

Age-associated immobilization progressing to total bedridden status causes reduced tissue perfusion and senile dermal atrophy. Pressure injuries (decubitus ulcers) developing secondary to sustained tissue compression completely compromise the protective integrity of the stratum corneum barrier. Field data demonstrate that pressure ulcers constitute a major clinical challenge among home-care recipients, with exudative wound swabs predominantly exhibiting colonization by multidrug-resistant nosocomial and opportunistic pathogens, primarily *Pseudomonas aeruginosa*, along with *Morganella morganii*, *Providencia stuartii*, *Acinetobacter baumannii/calcoaceticus* complex, *Escherichia coli*, and *Proteus mirabilis*. The loss of tissue integrity combined with secondary bacterial colonization creates an optimal niche for ectoparasitic infestation, while concurrently facilitating the deep-tissue penetration of helminths and protozoa across disrupted dermal and mucosal barriers. Moreover, prolonged stationary recumbency and the use of alternating-pressure air mattresses, coupled with localized heat accumulation and moisture retention,

foster a microclimate favorable to ectoparasites and disease vectors (Zeynudin et al., 2013; Kottner et al., 2018; EPUAP, 2019).

2.2. The Role of Fecal and Urinary Incontinence Alongside Absorbent Brief Usage in Modulating Pathogen Transmission Dynamics

Fecal and urinary involuntary discharge mandates the routine application of absorbent pads in bedridden care regimens. Constant moisture accumulation across the perianal and perineal zones, driven by continuous contact with excretions, leads to cutaneous maceration and loss of epidermal barrier efficiency. Evaluating the clinical-epidemiological scope demonstrates that perineal maceration is not merely a localized inflammatory issue. Rather, impaired epidermal cellular integrity significantly increases the success of tissue invasion by percutaneously acquired infective filariform larvae, such as *Strongyloides stercoralis*. Furthermore, pathogenic structures within fecal material namely *Giardia intestinalis* cysts, *Cryptosporidium* spp. oocysts, or *Enterobius vermicularis* eggs easily contaminate the hands of care personnel during suboptimal diaper changes. Poor hand sanitation subsequently facilitates the dissemination of these pathogens onto high-touch domestic surfaces like doorknobs, towels, and kitchen countertops, thereby converting a single patient's parasitic infection into a domestic cluster outbreak (Zeynudin et al., 2013; Shang et al., 2014; Kottner et al., 2018).

2.3. Dementia, Cognitive Deterioration, and Self-Care Deficiencies

Cognitive decline associated with dementia and neurodegenerative disorders manifested by deficits in orientation, memory, and reasoning compromises the consistent maintenance of personal hygiene. Aberrant behaviors focused on fecal-oral routes (e.g., scratching in response to perianal pruritus, hand-to-mouth movements, and touching domestic objects or food with unwashed hands) promote the harboring of parasitic agents beneath the fingernails. This dynamic triggers an ongoing autoinfection/retroinfection loop, sustaining a persistent host parasitic load (Cassell et al., 2018; Shinjyo et al., 2021).

2.4. Immunosenescence, Secondary Immunological Deficits, and Medication Patterns

Impairments in T-cell immunity secondary to age-related immunosenescence are deepened in geriatric cohorts by cancer chemotherapy, diabetes, chronic organ failures, and prolonged use of immunosuppressive agents or corticosteroids. The lack of routine prophylactic vaccination against influenza and pneumococcus in immunosenescent individuals further elevates systemic host susceptibility. When cellular immune responses are compromised, latent parasitic harborages within tissues can reactivate and spread systemically, culminating in fulminant hyperinfection crises. In summary, host-specific pathophysiological and behavioral vulnerabilities function as key determinants

that facilitate both tissue invasion and the transmission cycles of parasitic pathogens (Evering and Weiss, 2006; Herman and Tuttle, 2024).

3. Specific Parasitic Agents and Transmission Dynamics in the Home-Care Ecosystem

Categorizing the principal parasitic pathogens encountered within the home-care ecosystem based on their taxonomic traits, routes of transmission, and clinical sequelae is paramount for designing targeted preventive medicine interventions.

3.1. Ectoparasites

***Sarcoptes scabiei* and Crusted (Norwegian) Scabies:** In home-care cohorts comprising bedridden, cognitively impaired, or immunocompromised individuals, *Sarcoptes scabiei* spreads swiftly via close skin-to-skin contact and the common use of garments and bedding. Parasitological confirmation requires microscopic analysis of skin scrapings obtained from clinical lesions using 10% KOH solution to detect active mites, eggs, or scybala. Because compromised cellular immunity blunts characteristic hypersensitivity reactions and pruritus, clinical diagnosis is frequently delayed. This results in the development of Crusted (Norwegian) Scabies, an intensely contagious condition defined by extensive hyperkeratotic crusting filled with millions of parasites, which presents an epidemic threat to cohabiting relatives and care providers (Hewitt et al., 2015; Bae et al., 2020).

***Pediculus humanus* (Pediculosis Infestation):** Suboptimal personal hygiene, restricted access to sanitation facilities, and extended recumbency increase host susceptibility to infestations with body lice (*Pediculus humanus corporis*) and head lice (*Pediculus humanus capitis*). In addition, the mutual sharing of bedding textiles and personal items acts as a key factor fueling inter-individual transmission dynamics (Takano et al., 2004; Toghroli et al., 2022).

3.2. Gastrointestinal Protozoan Pathogens

***Giardia intestinalis* and *Cryptosporidium* spp.:** Transmission occurs via the fecal-oral route through contaminated drinking and utility water, foodstuffs, and compromised hand hygiene. Particularly among dependent and immunocompromised elderly individuals, mucosal colonization by cystic/oocystic and trophozoite stages disrupts the intestinal absorptive surface, precipitating chronic diarrhea. The resulting severe fluid and electrolyte loss further deepens dehydration and secondary malnutrition (Girotto et al., 2013; Ahmadiara and Hajimohammadi, 2017).

***Entamoeba histolytica* and *Blastocystis* spp.:** The transmission of these parasitic agents is driven by fecal contamination of patient-care items and hygiene lapses occurring during nursing care or diaper replacements. Operating

via direct tissue-invasion mechanisms, *Entamoeba histolytica* triggers the development of amebic dysentery and amebic liver abscesses; meanwhile, *Blastocystis* species typically follow a clinical course characterized by opportunistic gastrointestinal manifestations, most notably in immunocompromised cohorts (Siagian and Maryanti, 2021).

3.3. Opportunistic Protozoan Pathogens

***Toxoplasma gondii*:** Within home-care cohorts receiving oncological chemotherapy or immunosuppressive agents, compromised cellular immunity triggers the recrudescence of dormant tissue cysts (bradyzoites), which transition into rapidly disseminating tachyzoites. This reactivation frequently results in severe clinical manifestations, including central nervous system lesions, encephalitis, and irreversible neurological impairment (Belk et al., 2018; Dubey, 2021).

3.4. Helminthic Pathogens (Intestinal Parasites)

***Enterobius vermicularis* (Pinworm Infestation):** Perianal itching frequently triggers an ongoing autoinfection loop operating through the anal-hand-oral pathway. Parasitological detection via conventional fecal microscopy yields low diagnostic sensitivity; consequently, the microscopic identification of eggs laid during the female parasite's nocturnal perianal migration via the Graham method (cellophane tape test), applied immediately after waking, represents the benchmark diagnostic standard. The dispersal of infective eggs into ambient indoor dust and bed linens exacerbates environmental persistence, creating a pronounced risk of infection among care personnel (Wendt et al., 2019).

***Strongyloides stercoralis*:** Among home-care recipients subjected to long-term corticosteroid administration, rhabditiform larvae residing in the gut lumen swiftly differentiate into infective filariform larvae. As these infective larvae breach the intestinal mucosal barrier or perianal integument to enter systemic circulation, an autoinfective loop is triggered. Driven by underlying immunosuppression, this cascade may escalate into potentially fatal Hyperinfection Syndrome and widespread disseminated strongyloidiasis (Czeresnia and Weiss, 2022).

***Ascaris lumbricoides* and *Taenia* Species:** Transmission of these helminths is driven by fecal-oral dissemination, exposure to contaminated produce or soil, and the intake of inadequately cooked meats. Clinically, these parasitic infections can precipitate intestinal blockage, malabsorption syndromes, and secondary anemia (Gabriël et al., 2017).

A comprehensive understanding of pathogen transmission dynamics and clinical progression is essential for establishing targeted infection control and preventive measures aimed at disrupting transmission pathways.

4. Strategies for Breaking the Chain of Transmission: Prevention and Control Protocols

Interrupting parasitic transmission dynamics during home-based care necessitates embedding physical, chemical, and hygienic interventions within standardized operational procedures. Adopting such an integrative strategy is crucial for mitigating infectious risks and maintaining biosecurity for patients, caregivers, and cohabiting family members alike.

4.1. WHO Standard Hand Hygiene Protocols

In alignment with World Health Organization (WHO) guidelines, proper hand hygiene requires a minimum of 20–30 seconds of active friction both before and after engaging in patient contact, diaper replacements, wound care, or exposure to biological excretions and secretions. The cleansing process must emphasize anatomically critical yet commonly overlooked zones that harbor elevated contamination risks, such as subungual areas, fingertips, thumbs, and interdigital web spaces (WHO, 2006).

4.2. Soap Choice and Drying Protocol:

Given that solid bar soaps can support the survival of microbial agents and parasitic eggs on their outer surfaces, liquid soaps are recommended within household environments, while antiseptic formulations are preferred in professional care settings. Additionally, since damp conditions facilitate microbial multiplication, thorough hand drying must be accomplished using disposable paper towels immediately after washing (WHO, 2006).

4.3. Textile, Laundry, and Bedding Sanitation

High-Temperature Decontamination: Washing contaminated mattress linens, sheets, towels, and personal garments at temperatures of 60°C or higher, complemented by high-heat ironing, effectively neutralizes *Sarcoptes* mites, *Pediculus* lice species, and *Enterobius* eggs attached to textile fibers (Bloomfield et al., 2011).

Impermeable Bedding Covers: Ensuring that covers for static and air mattresses are liquid-impermeable and compatible with chemical disinfection prevents the soak-through of fecal or urinary excretions, thereby inhibiting the establishment of parasitic cyst reservoirs within mattress materials (Bloomfield et al., 2011).

4.4. Surface Disinfection and Caregiver Personal Protective Equipment Utilization

Adopting disposable gloves and protective gowns as an imperative protocol during handling of urine, stool, dermatological management, or pressure injury care is essential for infection control. Furthermore, high-frequency touch surfaces

across the domestic environment and medical equipment involved in routine care mandate systematic disinfection with suitable germicidal agents (Gershon et al., 2008).

5.1. Case Management and Continuous Monitoring Model

Healthcare professionals acting as case managers within home-based care coordinate targeted parasitic disease screening for individuals exhibiting chronic diarrhea, refractory perianal or systemic pruritus, unexplainable weight loss, or constitutional decline. Accordingly, diagnostic investigations are rapidly mobilized, including the Graham method (cellophane tape test) performed upon waking before washing for suspected *Enterobius vermicularis*, microscopic analysis of skin scrapings in 10% KOH for ectoparasitic pathologies (notably *Sarcoptes scabiei*), and direct and concentration wet-mount fecal microscopy when intestinal protozoa or helminths are suspected. Prompt field-based detection and tailored antiparasitic protocols effectively avert secondary sequelae, significantly reducing preventable hospital admissions (Yu et al., 2021).

5.2. Caregiver Burden, Education, and Family Support Strategies

Field data indicate that individuals providing home care are predominantly middle-aged and older females. While spouses and children constitute a significant proportion of primary caregivers, other family members also assume caregiving responsibilities throughout the care delivery process. The physical, psychological, and socio-economic burnout (caregiver burden) accumulated during caregiving leads to the neglect of personal and environmental hygiene standards, thereby elevating the risk of parasitic transmission. To mitigate these risks, structured continuing education programs covering parasitic transmission dynamics, hand hygiene, and personal protective protocols must be delivered to both informal (family) caregivers and professional home-care personnel. Supporting caregivers through a multidisciplinary and integrated framework ensures the sustainability of infection control in home-based healthcare, directly improving patient quality of life (Dowding et al., 2020).

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CHAPTER 23

Lymphatic Supermicrosurgery: From Lymphaticovenular Anastomosıs to Physiologic Reconstruction

İbrahim Kaan Yeşil[1]

[1] Stj. Dr., İstanbul Üniversitesi, İstanbul Tıp Fakültesi
ORCID: 0000-0001-9937-9177

1. Introduction

1.1. Lymphedema as a Failure of Lymphatic Transport

Lymphedema is a progressive failure of lymphatic transport rather than simple fluid accumulation. Primary lymphedema results from congenital or genetic abnormalities of lymphatic vessels or nodes, whereas secondary lymphedema follows acquired injury, most commonly lymphadenectomy, radiotherapy, trauma, or infection. Cancer treatment is the leading cause in industrialized countries, while lymphatic filariasis remains an important global contributor.

After breast cancer treatment, pooled upper-extremity lymphedema incidence has been estimated at 16.6%, increasing to 21.4% in prospective cohorts (DiSipio, Rye, Newman, & Hayes, 2013).

Lymphatic failure initiates a self-perpetuating process of protein-rich fluid accumulation, chronic CD4-positive T-cell inflammation, fibrosis, and adipose deposition, ultimately causing further deterioration of lymphatic function (Brown, Dayan, Kataru, & Mehrara, 2023). Recurrent cellulitis both results from and worsens this dysfunction.

This progression is central to surgical decision-making. Functional lymphatic collectors present early in the disease may later become sclerotic and unsuitable for reconstruction. Procedure selection must therefore reflect the remaining anatomy and function of the lymphatic system.

1.2. The Limits of Conservative Management

Complete decongestive therapy, including manual lymphatic drainage, compression, skin care, and exercise, remains the standard first-line treatment and is endorsed by the International Society of Lymphology (Executive Committee of the International Society of Lymphology, 2023). Although it reduces limb volume, symptoms, and infection risk, it does not restore lymphatic transport and requires continued adherence. Its role is therefore primarily compensatory rather than reconstructive.

1.3. The Emergence of Physiologic Surgery

Traditional lymphedema surgery focused on removing excess fibroadipose tissue. Physiologic surgery instead aims to restore or redirect lymphatic drainage.

Four procedures form the current reconstructive framework. Lymphaticovenular anastomosis (LVA) diverts lymph from functioning collectors into low-pressure veins. Vascularized lymph node transfer (VLNT) transfers vascularized lymphoid tissue, whereas vascularized lymphatic vessel and lymphatic tissue transfer (VLVT) provides lymphatic conduits without relying on nodal tissue. Immediate lymphatic reconstruction (ILR) restores

disrupted lymphatic drainage during oncologic surgery. This shift reflects a broader move from volume reduction toward restoration of lymphatic physiology.

The central question is whether impaired lymphatic function can be reconstructed rather than merely controlled. Current evidence supports this concept, but success depends on the residual lymphatic anatomy and its functional status. Modern lymphatic surgery is therefore increasingly imaging-guided and physiology-based, with procedure selection determined by residual lymphatic anatomy and function (Figure 1).

2. Evolution of Lymphatic Surgery: From Microsurgery to Supermicrosurgery

2.1. From Conventional Microsurgery to Submillimetre Vessels

Lymphaticovenous drainage predates modern supermicrosurgery. Lymphaticovenous anastomosis was first reported in the 1960s, but it has been performed consistently and successfully only since the advent of supermicrosurgery (Badash, Gould, & Patel, 2018). These early procedures established the principle of physiologic lymphatic drainage, but conventional microsurgery remained limited by vessel size: many functional lymphatic collectors suitable for bypass are substantially smaller than standard microsurgical vessels.

2.2. The Birth of Supermicrosurgery

Supermicrosurgery evolved through Koshima's work on increasingly small vessels and is defined as microneurovascular anastomosis of vessels and single nerve fascicles approximately 0.3–0.8 mm in diameter (Badash et al., 2018). In this chapter, the term refers broadly to surgery on submillimetre structures.

2.3. Why the Lymphatic System Became an Ideal Target

Lymphatic collectors are particularly suited to supermicrosurgery because of their small calibre, low-pressure flow, and network redundancy. Many collectors lie within the supermicrosurgical range, their low-pressure physiology reduces hemodynamic stress on the anastomosis, and the distributed lymphatic network allows multiple functional collectors to be bypassed through small incisions.

Koshima and colleagues subsequently applied supermicrosurgical lymphaticovenular anastomosis to extremity lymphedema, establishing supermicrosurgical lymphatic reconstruction as a practical therapeutic strategy (Badash et al., 2018).

3. Surgical Anatomy and Pathophysiology of the Lymphatic System

3.1. Microanatomy of Lymphatic Vessels

The lymphatic vasculature is hierarchically organized from initial lymphatics to precollectors and collecting vessels (Figure 2). Initial lymphatics are blind-ended capillaries lined by overlapping endothelial cells connected by discontinuous button-like junctions that permit entry of interstitial fluid and macromolecules. They drain into precollectors and subsequently into collecting lymphatic vessels, which are the operative targets of lymphaticovenular anastomosis.

Collecting vessels are divided by bicuspid valves into lymphangions, the functional pumping units of the lymphatic system. Their muscular walls generate rhythmic contractions through coordinated calcium-dependent electrical activity, while valve function maintains directional flow. Lymph propulsion depends on both intrinsic lymphangion contraction and extrinsic forces such as skeletal muscle activity, arterial pulsation, respiration, and external compression.

Superficial lymphatic drainage is regionally organized, with defined cutaneous territories draining toward specific nodal groups. This regional variability is clinically important because limited tracer injection may fail to identify otherwise functional collectors.

3.2. The Lymphatic–Venous Interface

Lymphaticovenular anastomosis depends on a favourable pressure gradient between the donor lymphatic and recipient venule. Normal lymphatic pumping can generate pressures sufficient to overcome venous afterload, but this capacity is reduced in lymphedema.

Anastomotic flow is therefore influenced by lymphatic contractility, recipient venous pressure, venous valve competence, and posture. This is particularly relevant in the lower extremity, where upright venous pressure opposes lymph-to-vein flow. Recipient venule selection is consequently a functional rather than purely technical decision and has been formalized in algorithmic approaches to LVA (Bianchi, Salgarello, Hayashi, Yang, & Visconti, 2022).

3.3. Pathophysiologic Progression and the Concept of Lymphatic Phenotype

Lymphatic dysfunction progresses through structural stages that directly affect reconstructive potential. The NECST classification describes collecting vessels as Normal, Ectasis, Contraction, or Sclerosis type (Mihara et al., 2012). Early ectatic vessels retain a lumen but show pressure-related changes, whereas progressive smooth-muscle dysfunction and collagen deposition lead to

contraction and ultimately sclerosis, with narrowing or obliteration of the lumen. Importantly, these changes may precede clinically evident lymphedema.

Chronic inflammation drives this progression. Lymphatic stasis promotes CD4-positive T-cell infiltration and Th2-mediated fibrosis and adipose deposition, creating a cycle of stasis, inflammation, and further lymphatic dysfunction (Brown et al., 2023). With advanced disease, tissue composition also changes substantially.

These changes define distinct reconstructive phenotypes. Patent and contractile collectors favour bypass procedures such as LVA. Sclerotic or obliterated collectors indicate loss of usable lymphatic conduits and may require tissue-based reconstruction. Advanced fibroadipose remodelling represents a predominantly tissue-composition problem that physiologic surgery alone cannot reverse. Preoperative imaging should therefore determine not only disease stage, but which elements of lymphatic anatomy and function remain reconstructable. The structural progression from a normal collector to advanced fibroadipose disease is illustrated in Figure 3.

4. Clinical Assessment and Staging of Lymphedema

4.1. Clinical Evaluation

Clinical assessment should establish both etiology and disease trajectory. Relevant factors include primary versus secondary disease, oncologic treatment and extent of lymphadenectomy or irradiation, symptom duration, cellulitis history, compression use, previous lymphatic or venous surgery, and body mass index. Venous pathology should also be considered, particularly before LVA, as proximal venous stenosis may alter recipient venule hemodynamics.

Examination should document the distribution and consistency of swelling, pitting, Stemmer sign, skin changes, lymphorrhea, and papillomatosis. Limb volume may be assessed by perometry, circumferential measurements, water displacement, or bioimpedance spectroscopy, but the same method should be used longitudinally because agreement between techniques is limited.

4.2. Clinical Staging and Its Limits

The International Society of Lymphology classifies lymphedema from Stage 0, representing subclinical transport impairment, to Stage III, characterized by advanced fibroadipose deposition and trophic skin changes (Executive Committee of the International Society of Lymphology, 2023). Stage I remains largely fluid-predominant and may improve with elevation, whereas Stage II progressively incorporates fat and fibrosis.

Clinical stage, however, describes the limb rather than the condition of individual lymphatic vessels, and histologic degeneration of collecting vessels

may already be present before lymphedema is clinically evident (Mihara et al., 2012). Clinical staging therefore defines disease severity but cannot determine reconstructability. Surgical planning requires direct assessment of residual lymphatic anatomy and function. The principal clinical and imaging-based classification systems are summarized in Table 1.

5. Imaging the Lymphatic System: From ICG Lymphography to Multimodal Mapping

Modern lymphatic surgery is fundamentally image-guided. Imaging defines the functional phenotype, identifies reconstructable vessels, guides incision placement, and increasingly determines the choice of operation.

5.1. Indocyanine Green Lymphography

Indocyanine green (ICG) lymphography provides real-time visualization of superficial lymphatic flow. Linear patterns indicate patent collectors, whereas splash, stardust, and diffuse dermal backflow represent progressive lymphatic dysfunction.

For surgical planning, ICG patterns also predict vessel quality. Splash and particularly diffuse regions are strongly associated with lymphosclerosis and are less suitable for LVA (Yamamoto, Yamamoto, Yoshimatsu, Narushima, & Koshima, 2017). ICG therefore combines diagnosis, functional staging, vessel selection, and incision planning. Its main limitation is shallow tissue penetration, especially when extensive dermal backflow obscures deeper functional collectors.

5.2. Lymphoscintigraphy

Lymphoscintigraphy provides whole-limb functional information, including transport kinetics, nodal uptake, delayed transit, and dermal backflow. Its main limitations are low spatial resolution and variable standardization, so its principal role is functional assessment and longitudinal documentation rather than precise operative mapping.

5.3. Magnetic Resonance Lymphangiography

Magnetic resonance lymphangiography (MRL) provides three-dimensional visualization of superficial and deep lymphatic anatomy while also characterizing edema and fibroadipose tissue. Because the relative contributions of fluid and fat can influence whether physiologic reconstruction, reductive surgery, or a combined approach is most appropriate, MRL complements functional imaging by defining both deep anatomy and tissue composition.

5.4. High-Frequency and Ultra-High-Frequency Ultrasound

High-frequency and ultra-high-frequency ultrasound provide direct visualization of lymphatic vessels at depths not accessible to ICG. Importantly, lymphatic ultrasound detects more vessels than ICG lymphography in lymphedematous limbs, including usable collectors in dermal-backflow regions and in advanced disease when ICG identifies none (Hara & Mihara, 2026). Ultrasound-based mapping therefore extends LVA to limbs in which ICG findings alone would preclude bypass.

5.5. Multimodal Mapping and Precision Lymphatic Surgery

No single imaging modality fully defines lymphatic anatomy, function, and tissue composition. ICG provides superficial functional mapping; ultrasound identifies and grades individual lymphatics and recipient venules; MRL visualizes deeper anatomy and tissue composition; and lymphoscintigraphy provides whole-limb functional context (van Heumen, Riksen, Bramer, van Soest, & Vasilic, 2023).

These modalities are therefore complementary rather than competing. A practical strategy uses ICG to define the functional phenotype, ultrasound to identify reconstructable vessels, MRL when deep anatomy or tissue composition may alter procedure selection, and lymphoscintigraphy when whole-limb transport assessment is required. Multimodal mapping shifts surgical planning from clinical-stage inference to direct assessment of what remains anatomically and functionally reconstructable—the basis of precision lymphatic surgery (Table 2).

6. Principles and Instrumentation of Lymphatic Supermicrosurgery

Lymphatic supermicrosurgery requires greater optical precision and tissue control than conventional microsurgery because collecting lymphatics lie within the supermicrosurgical size range and have thin, translucent walls. High-magnification microscopy, stable positioning, ultrafine forceps and needle holders, submillimetre dilators, contrasting backgrounds, and 11-0 or 12-0 nylon sutures are therefore standard.

Technical challenges include lumen collapse, poor wall visibility, size mismatch, tissue fragility, and venous reflux. Intraluminal nylon stenting reduces inadvertent back-wall suturing (Figure 4F), while careful atraumatic dissection and avoidance of thermal injury preserve vessel integrity. Venous reflux, in contrast, is managed primarily through careful recipient venule selection.

Supermicrosurgical proficiency requires dedicated training, for which synthetic, ex vivo, and live animal models are available (Badash et al., 2018).

Operator experience remains clinically relevant, as procedure selection may partly reflect surgical expertise rather than patient anatomy alone.

7. Lymphaticovenular Anastomosis

7.1. Physiologic Rationale

Lymphaticovenular anastomosis (LVA) bypasses an obstructed lymphatic pathway by connecting functioning distal collectors to nearby subdermal venules. It can be performed through small incisions, often under local anaesthesia, with limited physiologic burden.

Successful drainage requires three conditions: a flow-positive lymphatic collector, a recipient venule with sufficiently low pressure, and a patent anastomosis. Variation in these factors explains much of the heterogeneity in clinical outcomes.

7.2. Patient Selection: Beyond Early-Stage Disease

LVA should not be restricted solely by clinical stage. Advanced-stage patients may still benefit when functional collectors remain detectable (Cha et al., 2021), and ultrasound can identify such channels even when ICG lymphography shows none (Hara & Mihara, 2026).

Earlier disease generally produces better outcomes, but vessel quality is more important than stage alone. The key criterion is the presence of non-sclerotic, flow-positive collectors suitable for anastomosis (Gloviczki, Gloviczki, Mohos, & Oropallo, 2026).

7.3. Preoperative Mapping and Surgical Exposure

Preoperative planning should progress from functional phenotype to individual vessel selection. ICG lymphography identifies functional patterns and regions of severe dermal backflow, while ultra-high-frequency ultrasound locates candidate collectors, assesses wall quality, measures diameter and depth, and identifies suitable recipient venules.

Exposure is achieved through short skin incisions, with careful preservation of the collector and surrounding tissue. Final vessel suitability is confirmed intraoperatively according to lumen quality, wall thickness, translucency, contractility, and visible lymph flow.

7.4. Anastomotic Configurations

Four standard configurations are used: end-to-end, end-to-side, side-to-end, and side-to-side (Figure 4; Table 3). Selection depends mainly on lymphatic and venous calibre, flow direction, and the number of lymphatic channels requiring diversion.

End-to-end is the most commonly used configuration and is technically straightforward, but sacrifices proximal continuity of the lymphatic vessel (Bianchi et al., 2022). Side-to-end preserves proximal lymphatic flow, whereas end-to-side and side-to-side techniques are useful when vessel calibres differ or multiple lymphatic inflows must be accommodated.

More complex configurations, such as sequential and octopus-type constructions, allow several lymphatic channels to drain into limited venous recipients (Figure 4E). Overall, anastomotic geometry should follow lymphatic and venous flow dynamics rather than surgeon preference.

7.5. Assessment of Anastomotic Patency

Intraoperative patency is assessed by direct lymph flow, venous washout, and ICG transit across the anastomosis.

Clinical improvement does not always parallel volume reduction (Jonis et al., 2024), and the relationship between anastomotic patency and therapeutic benefit remains unresolved.

7.6. How Many Anastomoses?

Whether more anastomoses improve outcomes remains uncertain. Any apparent benefit of performing more LVAs may reflect greater residual lymphatic reserve rather than a true dose-response effect; vessel quality, lymph flow, recipient venous characteristics, anatomical location, and sustained patency are likely more important than absolute anastomosis number.

7.7. Upper Versus Lower Extremity

Upper- and lower-extremity lymphedema differ in venous anatomy and hydrostatic loading and should not be treated as equivalent conditions, as lower-extremity LVA is exposed to greater dependent venous pressure. Although pooled clinical improvement after physiologic surgery appears broadly similar in the two limbs (Hahn et al., 2025), outcomes should be reported separately for upper- and lower-extremity disease.

7.8. Postoperative Management

Postoperative compression is commonly used to maintain a favourable lymphatic-to-venous pressure gradient and support bypass flow. Early mobilization, infection prevention, and longitudinal assessment of limb volume and lymphatic function are also important. No standardised protocol, however, defines when compression can be reduced or discontinued, and establishing evidence-based criteria for compression withdrawal remains an important unresolved issue.

8. Vascularized Lymph Node Transfer

8.1. Concept

Vascularized lymph node transfer (VLNT) introduces functioning lymphoid tissue on its vascular pedicle into a lymphedematous region. Unlike LVA, it does not require a preserved native collector and therefore remains applicable when the local lymphatic network is severely damaged. The mechanisms, indications, ideal candidates, and principal limitations of the major physiologic procedures are compared in Table 4.

8.2. Proposed Mechanisms

Two principal mechanisms have been proposed. The pump or catchment hypothesis suggests that transferred nodes absorb interstitial lymph and drain it through the flap venous system, effectively creating a lymphaticovenous shunt. The lymphangiogenesis hypothesis proposes that transferred lymphoid tissue promotes the formation of new lymphatic connections through endogenous lymphangiogenic factors, including VEGF-C. This rationale underpinned a randomised trial combining VLNT with VEGF-C gene therapy (Rannikko et al., 2025). The relative importance of the two mechanisms remains unresolved.

8.3. Donor Sites and Trade-offs

Donor-site selection balances nodal yield, pedicle reliability, scar location, and risk of iatrogenic lymphedema. Common options include groin, submental, supraclavicular, lateral thoracic, and intra-abdominal donor sites. Flaps harvested from extremity drainage territories carry a potential risk of donor-limb lymphedema, whereas gastroepiploic and omental flaps avoid this risk at the cost of an intra-abdominal procedure. No donor site is universally superior.

8.4. Donor-Site Morbidity

Donor-site lymphedema is the principal specific complication of VLNT. Donor-site planning should therefore distinguish nodes draining the donor extremity from those draining the intended flap territory, so that extremity-draining nodes are preserved.

8.5. Recipient Site Selection

The optimal recipient site remains uncertain. Distal placement may benefit from gravity-assisted drainage, whereas proximal placement allows simultaneous scar release and decompression. Recipient-site selection therefore remains individualized.

8.6. Evidence

Meta-analytic evidence supports clinically meaningful benefit after VLNT. In upper-extremity lymphedema, pooled clinical improvement after VLNT numerically exceeded that after LVA, but with overlapping confidence intervals (Hahn et al., 2025).

However, the evidence base remains limited by retrospective designs, single-centre series, heterogeneity in technique and outcomes, and relatively sparse patient-reported data. VLNT is therefore established as a physiologic option, but comparative superiority over other reconstructive strategies remains uncertain.

9. Vascularized Lymphatic Vessel and Lymphatic Tissue Transfer

9.1. Rationale: Transferring the Conduit Rather Than the Node

Vascularized lymphatic vessel and lymphatic tissue transfer addresses loss or disruption of lymphatic conduits by transferring functioning lymphatic pathways rather than relying primarily on lymph nodes. This approach may restore continuity more directly while reducing donor-site risk associated with nodal harvest.

Its conceptual basis is lymph axiality: restoration of lymph flow depends on alignment between transferred lymphatic pathways and recipient lymphatic stumps. Lymph-interpositional flap transfer (LIFT) applies this principle by orienting a node-free vascularized flap across a lymphatic defect, and prospective data have shown higher rates of lymphatic flow restoration and lower postoperative lymphedema when lymphatic alignment is maintained (Yamamoto et al., 2021).

9.2. Techniques and Terminology

Several related procedures have emerged that transfer lymphatic-bearing tissue, with or without lymph nodes. Terminology remains inconsistent, with overlapping concepts described as vascularized lymphatic vessel transfer, lymphatic tissue transfer, LIFT, and lymphatic flow-through reconstruction.

9.3. Evidence and Unresolved Questions

Current evidence remains preliminary. A systematic review identified 12 studies including 182 patients: 59 underwent VLVT for established lymphedema, whereas 123 underwent immediate reconstruction for prevention. Outcomes were generally favourable, but techniques and outcome assessments were heterogeneous (Meroni, Martini, & Scaglioni, 2026).

Three limitations remain central: small sample sizes, inconsistent nomenclature, and limited long-term follow-up. Whether transferred lymphatic conduits remain functional over decades is unknown. These techniques therefore

represent a promising extension of physiologic reconstruction, but should not yet be regarded as equally established reconstructive options.

10. Combined and Personalized Physiologic Reconstruction

10.1. From Procedure Selection to Phenotype Matching

The central question is not whether LVA or VLNT is superior, but which component of lymphatic physiology remains reconstructable. Comparative evidence has not established clear superiority of either approach, and reported outcomes overlap substantially (Hahn et al., 2025).

Procedure selection should therefore follow phenotype. Patent, contractile, non-sclerotic collectors favour LVA, regardless of clinical stage. Partial collector degeneration may justify combining bypass with lymphatic tissue transfer, whereas advanced sclerosis without anastomosable vessels favours node- or lymphatic tissue-based reconstruction. When fibroadipose remodelling predominates, reductive surgery becomes necessary. In contrast, lymphatic transection during oncologic surgery represents an opportunity for immediate reconstruction before irreversible degeneration develops (Table 5; Figure 5).

10.2. Combining Physiologic and Reductive Surgery

Physiologic and reductive procedures address different components of lymphedema rather than competing indications. Liposuction removes excess fibroadipose tissue in chronic non-pitting disease, but it does not restore lymphatic transport and generally requires continued compression. Radical excisional procedures retain only a limited role, reserved for selected patients with severe refractory tissue overgrowth in end-stage disease, because of their substantial morbidity.

Combined strategies address both impaired drainage and fibroadipose deposition, an approach supported by recent international consensus recommendations (Executive Committee of the International Society of Lymphology, 2023; Masià et al., 2026).

11. Immediate Lymphatic Reconstruction and Prevention of Lymphedema

11.1. Concept

Immediate lymphatic reconstruction (ILR), also known as the lymphatic microsurgical preventive healing approach (LYMPHA), shifts treatment from established disease to prevention. During axillary or inguinofemoral lymphadenectomy, divided afferent lymphatics are identified and anastomosed to nearby venous branches before progressive lymphatic degeneration develops.

The technique was introduced by Boccardo and colleagues, whose single-centre experience now extends to 15 years of follow-up (Boccardo et al., 2026).

11.2. Evidence for Prevention

Current evidence supports a substantial reduction in short- to medium-term lymphedema risk after ILR. A meta-analysis of 42 studies including 4,539 patients reported a pooled odds ratio of 0.25 in favour of ILR (Du, Fu, & Liu, 2025).

11.3. The Durability Problem

Long-term durability remains uncertain. Meta-analytic data show attenuation of benefit as follow-up lengthens, with the pooled odds ratio rising from 0.11 within the first year to 0.41 at two years or more (Du et al., 2025).

However, the longest single-centre dataset reported sustained protection over 15 years, with markedly lower lymphedema incidence after LYMPHA than in corresponding non-LYMPHA groups (Boccardo et al., 2026). Attenuation in pooled data alongside sustained protection in this series prevents a firm conclusion regarding permanent prevention.

The most defensible interpretation is that ILR reduces and may delay lymphedema during the follow-up periods studied most extensively, while lifelong protection remains unproven. Resolving this uncertainty will require adequately powered randomised trials with follow-up extending beyond five years. Regardless of the final durability estimate, ILR represents an important shift in lymphatic surgery from treating established dysfunction toward preventing failure at the time of injury.

12. Defining Surgical Success: Outcomes and Patient-Reported Measures

Limb-volume reduction remains the most frequently reported outcome, but correlates poorly with patient experience: after LVA, function can improve and compression use can be reduced or stopped without a significant change in limb volume (Jonis et al., 2024). Surgical success should therefore be assessed across several complementary domains.

Volumetric outcomes remain important but should preferably be expressed as relative change or body-size-adjusted indices because absolute measurements are influenced by body habitus and natural temporal variation. Infection outcomes are particularly clinically relevant, as reduction in cellulitis is among the best-supported benefits of microsurgical treatment for lower-extremity lymphedema (Gloviczki et al., 2026). Compression dependence, including daily duration, garment class, and complete discontinuation, should also be reported because it directly reflects treatment burden.

Patient-reported outcomes should be measured with validated disease-specific instruments. Imaging and anastomotic measures, including repeat ICG lymphography, lymphoscintigraphy, and patency assessment, provide complementary evidence of physiologic function.

Outcome reporting remains insufficiently standardised. A modified Delphi process from the Society of Lymphatic Surgery leadership has proposed a minimum standard set of outcome measures for lymphatic surgery (Fanning et al., 2025). Wider adoption of standardised outcome sets is essential for meaningful comparison across studies.

13. Current Evidence, Limitations, and Controversies

The overall evidence supports clinical benefit from physiologic lymphatic surgery, but its quality remains limited. An umbrella review of 26 systematic reviews found no randomised controlled trials of microsurgical treatment for lower-extremity lymphedema. Evidence for volume or circumference reduction was rated low to very low quality, whereas reduction in cellulitis reached moderate-quality evidence; quality of life and symptoms generally improved across reviews (Gloviczki et al., 2026).

Several limitations recur throughout the literature: heterogeneous diagnostic criteria, multiple poorly aligned staging systems, variable operative techniques, inconsistent outcome measures, short follow-up, selection and publication bias, and limited randomised evidence. These problems limit direct comparison between procedures and complicate interpretation of pooled estimates.

Randomised trials are beginning to address these gaps. Interim randomised data comparing LVA with conservative therapy suggest improvements in selected functional outcomes and reduced compression dependence even when limb volume does not change significantly (Jonis et al., 2024), but larger trials with long-term follow-up are still required.

At present, claims of superiority between LVA, VLNT, and other reconstructive procedures are not justified. Evidence is most consistent for reductions in infection burden, while improvements in symptoms and quality of life are also frequently reported. Procedure selection therefore remains primarily guided by lymphatic phenotype, imaging findings, tissue composition, and institutional expertise (Table 6; Table 7).

14. Robotic-Assisted Lymphatic Supermicrosurgery

14.1. Rationale and Platforms

Robotic assistance is particularly suited to lymphatic supermicrosurgery because it addresses limitations of human motor control at the submillimetre scale. Dedicated microsurgical robots, such as the MUSA system used in the first

randomised trial of robot-assisted LVA, provide motion scaling and tremor filtration (van Mulken et al., 2020), although current platforms lack haptic feedback.

14.2. Clinical Evidence

Early clinical evidence demonstrates feasibility but not clear superiority over manual surgery. In the first randomised pilot trial of robot-assisted versus manual LVA, robotic anastomosis required substantially more time, while both groups achieved patent anastomoses and similar short-term improvements in Lymph-ICF scores (van Mulken et al., 2020).

14.3. Limitations

Robotic anastomosis remains slower than manual supermicrosurgery, and lack of haptic feedback, setup requirements, capital and procedural costs, and limited access remain major barriers.

Current evidence therefore supports robotic assistance as a technically feasible platform with potential precision and ergonomic advantages rather than a clinically superior replacement for manual supermicrosurgery. Comparative effectiveness, cost-effectiveness, and the specific procedures in which robotic assistance provides meaningful clinical advantage remain unresolved.

15. Emerging Imaging and Navigation Technologies

Photoacoustic lymphangiography combines optical contrast with greater imaging depth, enabling three-dimensional visualization of lymphatics and adjacent veins, but it remains a research-stage technique available in few centres.

Augmented reality navigation and artificial intelligence are similarly promising but immature. Both remain experimental, and no AI system for intraoperative lymphatic vessel identification has yet been prospectively validated.

16. Future Directions: Toward Precision and Regenerative Lymphatic Surgery

Four trends are likely to shape the next phase of lymphatic surgery (Figure 6).

Earlier. Management is moving from established disease toward subclinical detection and prevention at the time of lymphatic injury. The key unresolved question is whether immediate reconstruction prevents lymphedema permanently or merely delays its onset, which requires long-term randomised evidence.

Smaller. Supermicrosurgery has extended reconstruction to submillimetre vessels, while robotic platforms may further improve precision and reproducibility at this scale.

Smarter. Multimodal imaging and phenotype-based selection are replacing stage-based decision-making. The long-term objective is to tailor surgery to measurable features of each patient's lymphatic anatomy, function, and tissue composition.

More physiologic. Reconstruction is progressing from bypass toward preservation and regeneration. However, a double-blind phase II trial of VEGF-C gene therapy combined with VLNT found no significant benefit over VLNT alone for the primary endpoints of oedema volume, quality of life, or lymphoscintigraphy (Rannikko et al., 2025). Regenerative strategies therefore remain promising but unproven, and approaches such as molecular modulation and lymphatic tissue engineering remain largely preclinical.

17. Conclusions

Lymphatic supermicrosurgery has transformed lymphedema surgery from primarily reductive treatment toward physiologic reconstruction. LVA, VLNT, vascularized lymphatic vessel and lymphatic tissue transfer, and ILR address different components of lymphatic failure. Current evidence supports meaningful clinical benefit, particularly in reducing cellulitis and improving patient-reported outcomes, but comparative superiority between procedures remains unproven.

Patient selection should therefore be based on lymphatic phenotype rather than clinical stage alone. The relevant question is whether functional collectors remain, whether they can be identified, and whether the dominant pathology is impaired outflow, loss of lymphatic conduits, or fibroadipose remodelling. ICG lymphography, ultra-high-frequency ultrasound, and magnetic resonance lymphangiography provide complementary information for answering these questions.

The future of the field lies in prevention, multimodal imaging, phenotype-guided reconstruction, robotic assistance, and regenerative strategies. Progress, however, will depend less on adding new procedures than on producing adequately powered randomised trials with long-term follow-up and standardised outcome reporting. These advances are essential for determining which reconstructive strategy is most appropriate for each lymphatic phenotype.

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Tables

Table 1. Clinical and Imaging-Based Classification Systems for Lymphedema

System	Basis	Categories	Principal strength	Principal limitation
ISL staging (Executive Committee of the International Society of Lymphology, 2023)	Clinical appearance of the limb	Stage 0 (latent/subclinical), I (pitting, reduces with elevation), II (permanent solids; pitting early, absent late), III (lymphostatic elephantiasis); severity graded minimal >5–<20%, moderate 20–40%, severe >40% volume increase	Universal reference standard; simple; internationally adopted	Describes the limb, not the lymphatic vessels; a single limb may exhibit more than one stage in different territories
ICG dermal backflow patterns	Extent and pattern of ICG dermal backflow	Linear; splash; stardust; diffuse	Strongly predicts intraoperative lymphosclerosis (diffuse region OR 1441; Yamamoto et al., 2017)	Depth-limited; severe backflow obscures deeper collectors
NECST vessel classification (Mihara et al., 2012)	Histology of the collecting vessel	Normal, Ectasis, Contraction, Sclerosis type	The only system that classifies the operative target itself	Requires direct assessment of the collecting vessel

Note. ICG = indocyanine green; ISL = International Society of Lymphology; NECST = Normal, Ectasis, Contraction, Sclerosis type; OR = odds ratio.

Source: Compiled by the author from Executive Committee of the International Society of Lymphology (2023), Mihara et al. (2012) and Yamamoto et al. (2017).

Table 2. Imaging Modalities in Lymphatic Supermicrosurgery

Modality	Anatomic detail	Functional information	Effective depth	Principal surgical role	Key limitation
ICG lymphography	High for superficial collectors	High (real-time flow, dermal backflow patterns)	Superficial (~1–2 cm)	Disease phenotyping, severity staging, identification of regions to avoid, incision planning, intraoperative navigation and patency check	Signal from dermal backflow obscures deeper vessels; useless in ICG allergy; sensitivity falls as disease advances
High- and ultra-high-frequency ultrasound	Very high for individual vessels	Moderate	Deeper than ICG; detects vessels invisible in dermal-backflow regions	Vessel selection, diameter and depth measurement, simultaneous recipient venule identification	Highly operator-dependent; requires dedicated training and equipment
Magnetic resonance lymphangiography	High, three-dimensional, superficial and deep	Moderate	Whole limb	Global mapping, deep system assessment, fluid-versus-fibroadipose discrimination, selection between physiologic and reductive surgery	Cost, availability, scan time; contrast protocols vary
Lymphoscintigraphy	Low	High (whole-limb transport, nodal uptake)	Whole body	Functional diagnosis, documentation, follow-up, medicolegal confirmation	Poor spatial resolution; limited standardization

Note. ICG = indocyanine green.

Source: Compiled by the author, drawing on van Heumen et al. (2023), Yamamoto et al. (2017) and Hara and Mihara (2026).

Table 3. Technical Configurations of Lymphaticovenular Anastomosis

Configuration	Construction	Typical indication	Reported advantage	Caveat	Relative frequency
End-to-end	Divided lymphatic to divided venule	Lymphatic and venule of comparable calibre	Technically simplest; the reference configuration	Sacrifices proximal lymph flow; occlusion converts partial to complete obstruction	82% of 1,000 consecutive LVAs (Bianchi et al., 2022)
End-to-side	Divided lymphatic into the side wall of a larger vein	Venule calibre substantially exceeds lymphatic	Uses a larger, more robust recipient	Higher reflux risk with a large recipient	11%
Side-to-end	Side of an intact lymphatic to a divided venule	Preservation of proximal lymph flow desired	Preserves proximal lymph flow	Limited comparative data	5%
Side-to-side	Side of lymphatic to side of vein	Neither vessel should be divided; multiple flows into one venule	Preserves both native pathways	Technically demanding; small evidence base	2%
Complex multi-lymphatic configurations (e.g., sequential, octopus)	Multiple lymphatics diverted into a single venule by combined configurations	Available lymphatics outnumber available venules	More lymph flows diverted per available venule	Longer operative time; heterogeneous nomenclature; limited comparative data	Not systematically reported

Note. LVA = lymphaticovenular anastomosis. Configuration selection should follow three measured variables — lymphatic calibre, venule calibre, and the number of lymph flows requiring diversion — rather than surgeon preference.

Source: Prepared by the author; relative frequencies from Bianchi et al. (2022).

Table 4. Physiologic Surgical Procedures for Lymphedema

Procedure	Mechanism	Ideal candidate	Advantages	Limitations	Evidence maturity
Lymphaticovenular anastomosis (LVA)	Diverts lymph from functioning collectors into low-pressure venules, bypassing the obstruction	Demonstrable patent, non-sclerotic, flow-positive collectors — at any ISL stage	Minimally invasive; frequently under local anaesthesia; no donor site; low morbidity; rapid early improvement	Requires salvageable collectors; posture-dependent hemodynamics in the lower limb	Large observational base; one open-label RCT with interim results
Vascularized lymph node transfer (VLNT)	Contested: lymphaticovenous pump/catchment effect and/or induced lymphangiogenesis	Advanced collector loss with no anastomosable vessels; scarred axilla requiring release; recurrent cellulitis	Independent of native collector patency; permits simultaneous scar release	Free tissue transfer with attendant morbidity; risk of iatrogenic donor-site lymphedema; mechanism unresolved; recipient site unresolved	Large observational base; multiple meta-analyses; no head-to-head RCT
Vascularized lymphatic vessel / tissue transfer (VLVT, LIFT)	Transfers lymphatic conduits, with or without nodes, aligned to the recipient lymphatic axis	Segmental loss of lymphatic continuity; concurrent soft-tissue defect requiring flap reconstruction	Reduced donor-site lymphedema risk; may restore continuity rather than create a shunt; LIFT requires no anastomosis	Small evidence base (12 studies, 182 patients); unsettled terminology; long-term durability unknown	Early; promising but preliminary
Immediate lymphatic reconstruction (ILR / LYMPHA)	Anastomoses lymphatics transected during lymphadenectomy to an adjacent vein at the index operation	Patients undergoing axillary or inguinofemoral lymphadenectomy, particularly with planned regional nodal irradiation	Operates on normal, non-degenerated lymphatics; adds limited operative time	Efficacy attenuates with longer follow-up; requires microsurgical availability at the oncologic operation	Multiple meta-analyses; long-term randomised data awaited

Note. ILR = immediate lymphatic reconstruction; ISL = International Society of Lymphology; LIFT = lymph-interpositional flap transfer; LYMPHA = lymphatic microsurgical preventive healing approach; RCT = randomised controlled trial.

Source: Compiled by the author, drawing on Du et al. (2025), Gloviczki et al. (2026), Hahn et al. (2025), Jonis et al. (2024), Meroni et al. (2026) and Yamamoto et al. (2021).

Table 5. Phenotype-Based Framework for Procedure Selection

Lymphatic phenotype	How it is identified	Dominant problem	Potential approach
Functional patent collectors	Linear ICG pattern; normal-to-ectatic vessels on UHFUS; lymph flow at exploration	Outflow obstruction	LVA
Partially damaged network	Mixed linear and splash/stardust ICG; ectatic and contraction-type vessels on UHFUS	Mixed conduit and transport dysfunction	LVA, with VLNT considered as an adjunct or staged procedure
Advanced collector sclerosis	Diffuse ICG backflow with no linear pattern; sclerosis-type vessels on UHFUS; no anastomosable vessel at exploration	Loss of functional conduits	VLNT or vascularized lymphatic vessel/tissue transfer
Marked fibroadipose remodelling	Non-pitting limb; fat-predominant pattern on MR	Structural tissue excess rather than fluid	Physiologic reconstruction combined with, or preceded by, reductive surgery
Lymphatics transected during oncologic surgery	Intraoperative ICG or blue dye identification of divided afferent lymphatics	Imminent, not yet established, lymphatic failure	ILR / LYMPHA
Segmental defect with loss of lymphatic continuity	Imaging-demonstrated gap with viable lymphatics proximal and distal; concurrent soft-tissue defect	Loss of conduit continuity	Vascularized lymphatic vessel/tissue transfer; LIFT respecting lymph axiality

Note. ICG = indocyanine green; ILR = immediate lymphatic reconstruction; LIFT = lymph-interpositional flap transfer; LVA = lymphaticovenular anastomosis; LYMPHA = lymphatic microsurgical preventive healing approach; MR = magnetic resonance; UHFUS = ultra-high-frequency ultrasound; VLNT = vascularized lymph node transfer. This framework is a synthesis of the evidence reviewed in this chapter and is offered as a reasoning structure, not as a validated algorithm.

Source: Prepared by the author, drawing on Mihara et al. (2012), Yamamoto et al. (2017), Yamamoto et al. (2021) and Hara and Mihara (2026).

Table 6. Contemporary Evidence for Physiologic Lymphatic Reconstruction (2025–2026)

Study	Design	N	Intervention	Main outcome	Stated limitations
Hahn et al. (2025)	Systematic review and meta-analysis	52 studies	LVA and VLNT	Clinical improvement 36.46% (UEL) and 34.16% (LEL); LVA 29.44% versus VLNT 41.66% in UEL	Overlapping confidence intervals; heterogeneous outcome definitions
Meroni et al. (2026)	Systematic review	12 studies, 182 patients	Vascularized lymphatic vessel transfer	Objective and subjective improvement in most treated patients; no cases of worsening	Small pooled N; outcome assessment varied significantly across studies
Gloviczki et al. (2026)	Umbrella systematic review	26 systematic reviews, 14 meta-analyses	Microsurgical treatment of lower extremity lymphedema	Volume reduction 34.16–46.8% (Evidence C); cellulitis reduction (Evidence B); ILR risk difference −30.3%	No randomised controlled trials identified; substantial overlap of primary studies
Du et al. (2025)	Meta-analysis	42 studies, 4,539 patients	ILR	Pooled OR 0.25 (95% CI 0.17–0.36); OR 0.11 at <1 year versus 0.41 at ≥2 years (p = .003)	Publication bias in comparative studies (Egger p = .032); I^2 = 51%
Boccardo et al. (2026)	Single-centre cohort, 15-year follow-up	500	LYMPHA versus no LYMPHA	2.5% versus 45% with radiotherapy; lymphedema-free probability 0.978 versus 0.587 (p < .001)	Single centre; non-randomised
Rannikko et al. (2025)	Randomised, double-blind, placebo-controlled phase II	39	Adenoviral VEGF-C (Lymfactin) + VLNT versus placebo + VLNT	Positive effect of VLNT in both arms; no significant between-group difference in primary endpoints	Small sample; 12-month endpoint

Note. CI = confidence interval; ILR = immediate lymphatic reconstruction; LEL = lower extremity lymphedema; LVA = lymphaticovenular anastomosis; LYMPHA = lymphatic microsurgical preventive healing approach; OR = odds ratio; UEL = upper extremity lymphedema; VEGF-C = vascular endothelial growth factor C; VLNT = vascularized lymph node transfer.

Source: Compiled by the author from Boccardo et al. (2026), Du et al. (2025), Gloviczki et al. (2026), Hahn et al. (2025), Meroni et al. (2026) and Rannikko et al. (2025).

Table 7. Current Controversies and Research Priorities

Question	Current state of uncertainty	What would resolve it
What is the optimal number of LVAs?	Unresolved; anastomosis count may be confounded by residual lymphatic reserve, vessel quality, and sustained patency	Prospective study stratifying by vessel quality rather than count, with imaging-confirmed patency as a covariate
Which anastomotic configuration is best?	No universal standard; end-to-end predominates by frequency (82% in a series of 1,000 LVAs), and comparative data between configurations are limited	Randomised comparison stratified by vessel and venule calibre and by disease stage
LVA or VLNT?	Phenotype-dependent. Meta-analytic point estimates favour VLNT numerically but with overlapping confidence intervals; no randomised comparison exists	Head-to-head randomised trial with phenotype stratification and a core outcome set
Does anastomotic patency drive clinical benefit?	Unresolved. Clinical and quality-of-life improvement can occur without proportional volume reduction	Longitudinal imaging of patency paired with PROs and volumetry in the same cohort
What is the optimal postoperative compression protocol?	No standard exists; criteria for reduction or cessation have not been established	Randomised comparison of compression protocols after LVA, with limb volume and QoL co-primary
Does ILR prevent or merely delay lymphedema?	Promising but unresolved. Short-term effect sizes are large, but the pooled effect attenuates with follow-up duration, while a 15-year single-centre series shows sustained benefit	Completed randomised trials reported at ≥5 years
How should surgical success be defined?	Not standardised. Limb volume correlates poorly with patient-reported impact	Adoption of the proposed minimum outcome set and a validated core outcome set across trials
What is the role of robotics?	Feasible in a randomised pilot trial; anastomosis remains slower than manual; cost-effectiveness not established	Randomised comparison powered for clinical outcome, plus a formal economic evaluation
Can regenerative approaches augment surgery?	Uncertain. A phase II randomised trial of VEGF-C gene therapy with VLNT found no between-group difference	Larger randomised trials with mechanistic endpoints; further preclinical work on delivery and dosing

Note. ILR = immediate lymphatic reconstruction; LVA = lymphaticovenular anastomosis; PRO = patient-reported outcome; QoL = quality of life; VEGF-C = vascular endothelial growth factor C; VLNT = vascularized lymph node transfer.

Source: Compiled by the author, drawing on Bianchi et al. (2022), Boccardo et al. (2026), Du et al. (2025), Fanning et al. (2025), Gloviczki et al. (2026), Hahn et al. (2025), Jonis et al. (2024), Rannikko et al. (2025) and van Mulken et al. (2020).

Figure Legends

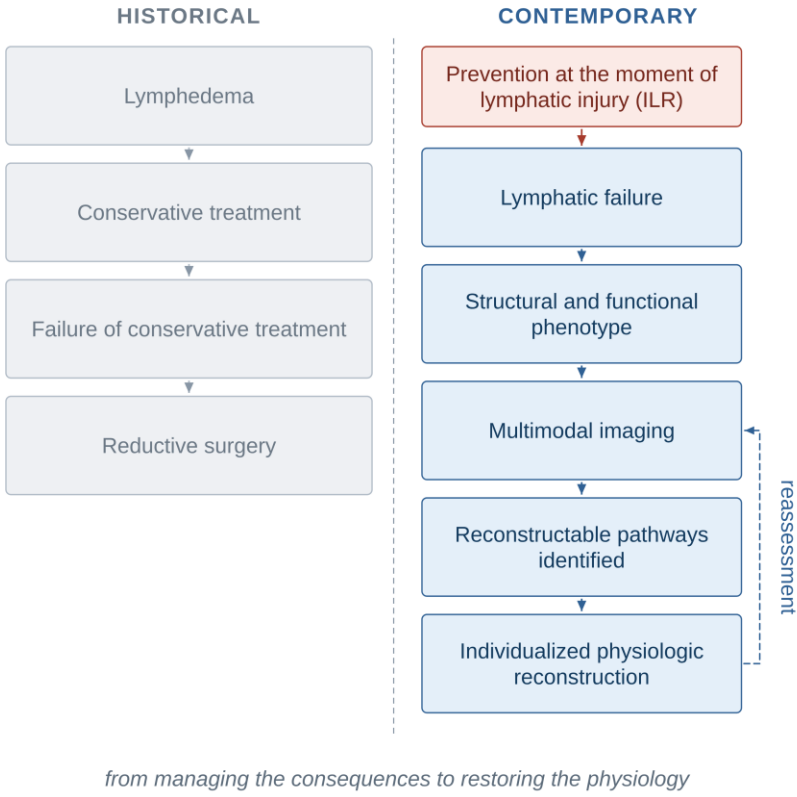


Figure 1. *Evolution from conventional lymphedema treatment to physiologic lymphatic reconstruction.* The historical paradigm proceeded linearly from diagnosis through conservative management to reductive surgery, with surgery framed as a last resort that removed the product of lymphatic failure without addressing the failure itself. The contemporary paradigm is iterative and imaging-driven: lymphatic failure is characterized as a structural and functional phenotype, that phenotype is defined by multimodal imaging, reconstructable lymphatic pathways are identified, and an individualized physiologic reconstruction is selected — with prevention at the moment of oncologic injury now positioned upstream of the entire sequence.

Source: Original figure prepared by the author.

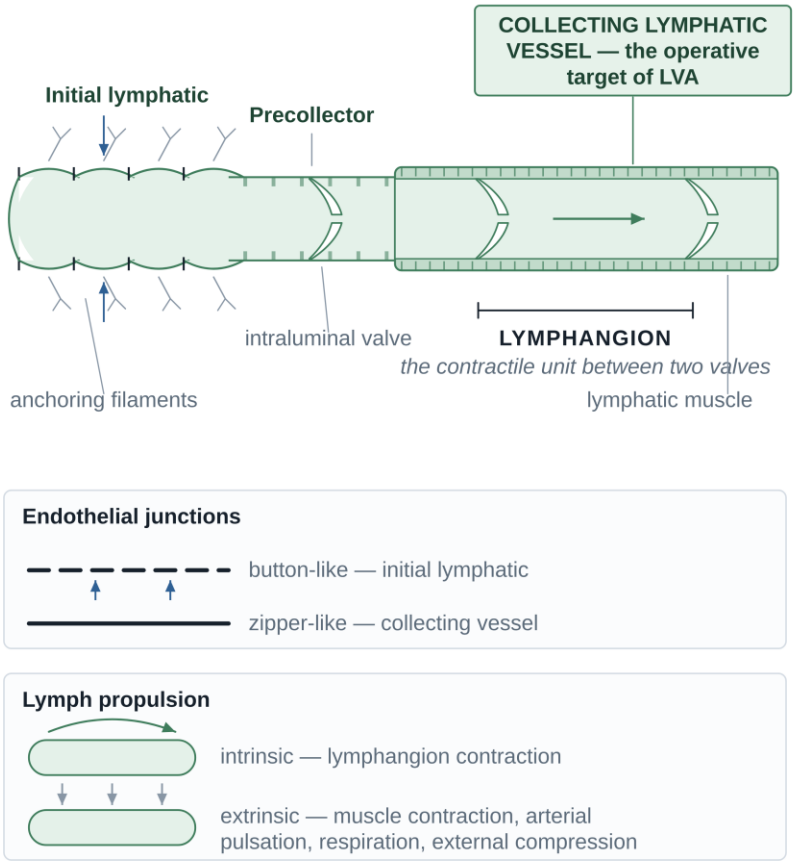


Figure 2. *Surgical microanatomy of the lymphatic collector and the lymphangion.* Interstitial fluid enters blind-ended initial lymphatics through overlapping endothelial flaps anchored by discontinuous button-like junctions and tethered to the surrounding matrix by anchoring filaments. Fluid passes through valved precollectors into collecting lymphatic vessels, whose wall carries a layer of lymphatic muscle cells and whose lumen is divided by bicuspid intraluminal valves into contractile units termed lymphangions. The collecting lymphatic vessel — not the initial lymphatic or the dermal plexus — is the operative target of lymphaticovenular anastomosis.

Source: Original figure prepared by the author.

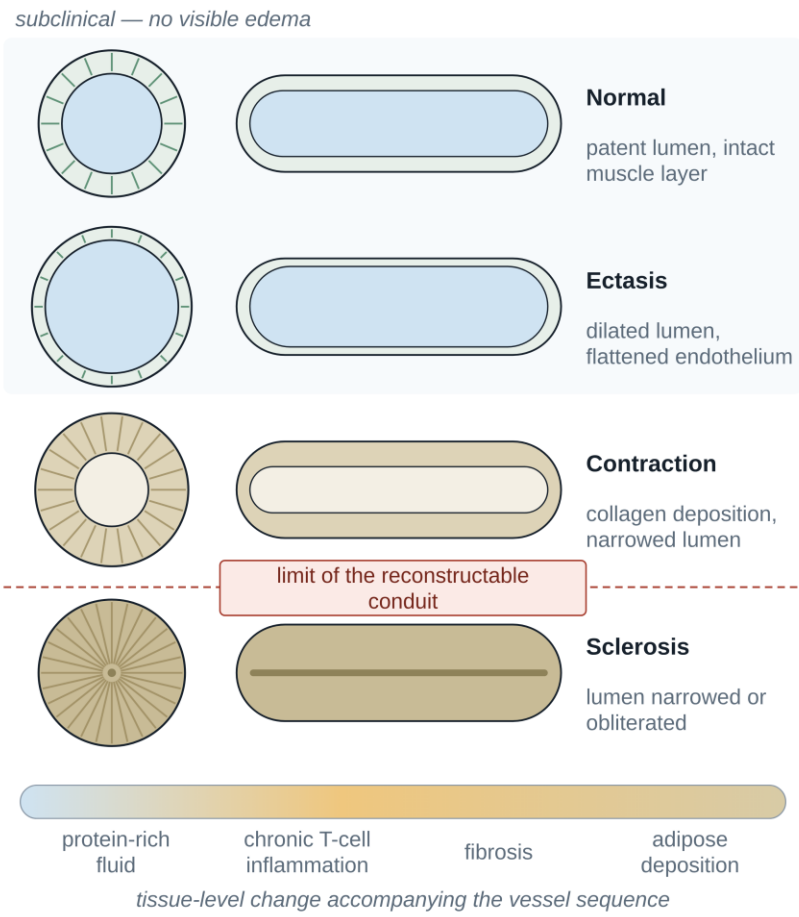


Figure 3. *Progression from the normal lymphatic collector to fibroadipose lymphedema.* Four panels illustrate the NECST sequence and its tissue consequences. In the normal vessel, the lumen is patent and the muscle layer intact. In the ectasis type, raised endolymphatic pressure dilates the lumen and flattens the endothelium. In the contraction type, lymphatic muscle cells adopt a synthetic phenotype and deposit collagen, thickening the wall. In the sclerosis type, fibrous elements predominate, transport capacity is lost, and the lumen is narrowed or obliterated. A parallel band beneath the panels shows the corresponding tissue-level change from fluid accumulation, through chronic T-cell-mediated inflammation, to fibrosis and adipose deposition. Histologic changes begin before clinically apparent lymphedema, and the horizontal dashed line marks the point beyond which no anastomosable conduit remains.

Source: Original figure prepared by the author.

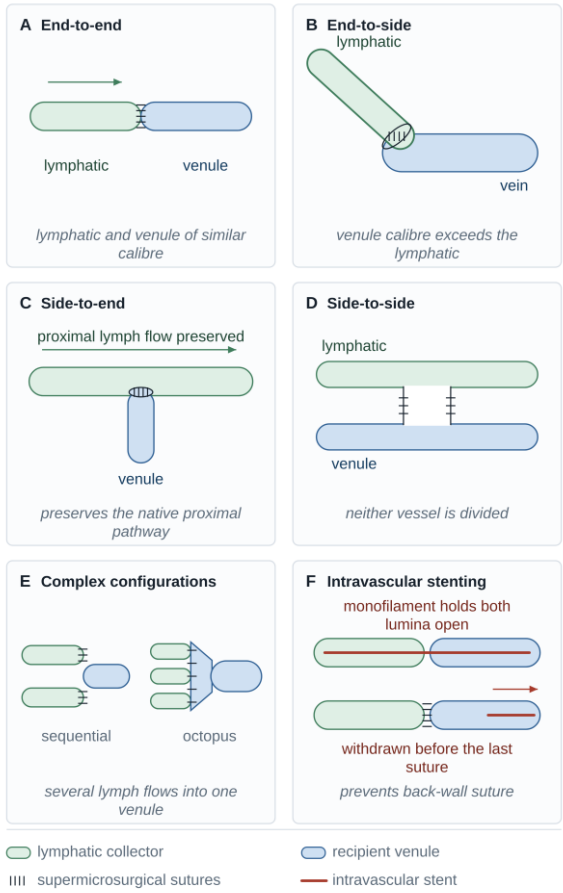


Figure 4. *Configurations of lymphaticovenular anastomosis.* (A) End-to-end: the divided lymphatic is sutured to a divided venule; the most frequently performed configuration but one that sacrifices proximal lymph flow. (B) End-to-side: the divided lymphatic is inset into the side wall of a larger vein. (C) Side-to-end: a divided venule is anastomosed to the side of an intact lymphatic, preserving the native proximal pathway. (D) Side-to-side: both vessels remain in continuity. (E) Complex configurations divert multiple lymph flows into a single recipient venule, illustrated here by a sequential construction and an octopus-type intussusception. (F) Intravascular stenting, in which a temporary nylon monofilament maintains the lumen and prevents inadvertent back-wall suture.

Source: Original figure prepared by the author.

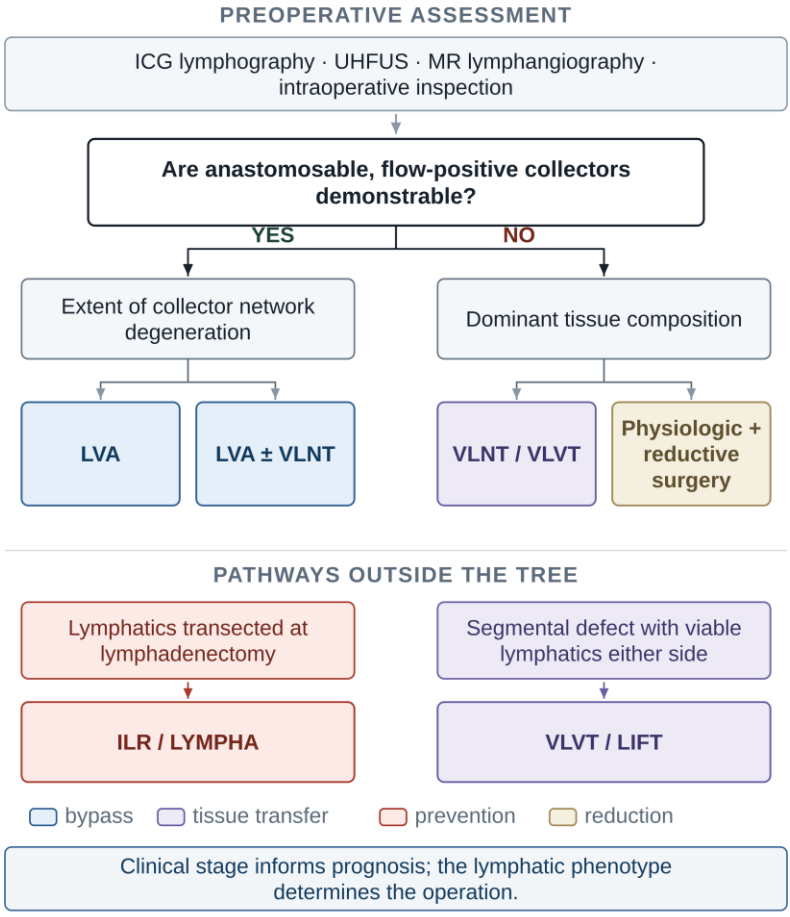


Figure 5. *Conceptual phenotype-based decision framework for physiologic lymphatic reconstruction.* Procedure selection follows from the lymphatic phenotype demonstrated on multimodal imaging and confirmed at exploration, rather than from clinical stage alone. Patent, contractile collectors indicate an outflow problem amenable to lymphaticovenular anastomosis at any stage. A partially degenerated network supports bypass with vascularized lymph node transfer as adjunct or staged procedure. Advanced collector sclerosis with no anastomosable vessel removes bypass as an option and indicates node or lymphatic tissue transfer. Fibroadipose predominance requires reductive surgery in combination with physiologic reconstruction. Lymphatics transected during oncologic surgery indicate immediate lymphatic reconstruction. Segmental loss of continuity indicates vascularized lymphatic vessel or tissue transfer respecting lymph axiality.

Source: Original figure prepared by the author.

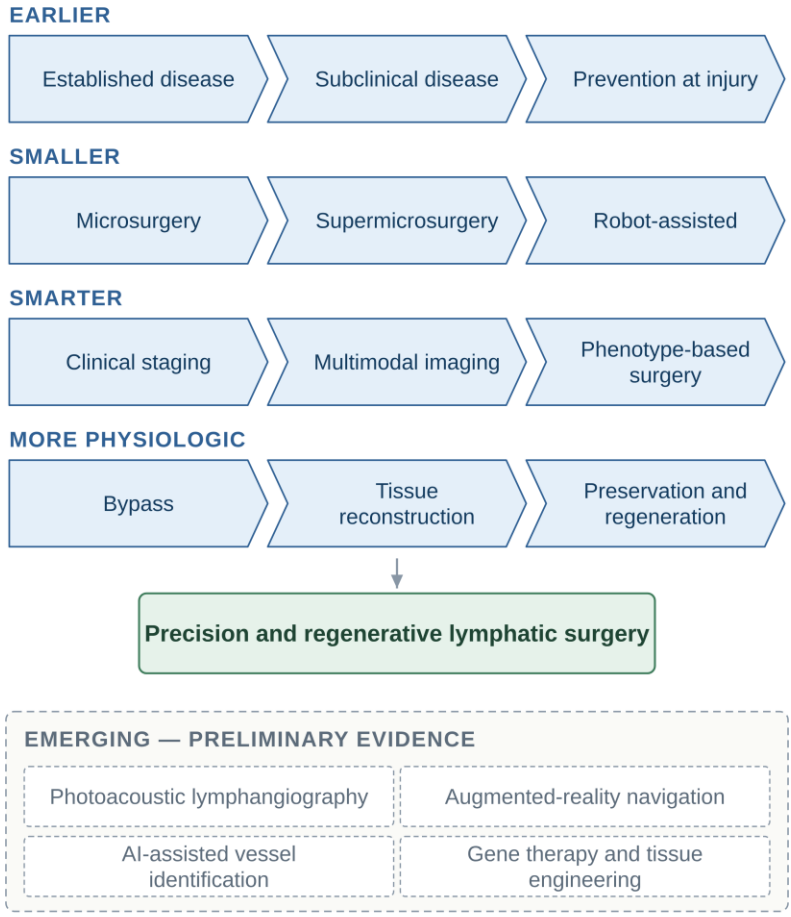


Figure 6. *Future of lymphatic supermicrosurgery.* Four converging vectors define the direction of the field. *Earlier:* the point of intervention moves from established disease, to subclinical disease detected before symptoms appear, to prevention at the moment of surgical injury. *Smaller:* from conventional microsurgery, to supermicrosurgery on submillimetre vessels, to robot-assisted work at and below that scale. *Smarter:* from clinical staging, to multimodal imaging, to phenotype-based procedure selection, with artificial-intelligence-assisted vessel identification and augmented-reality navigation as emerging adjuncts. *More physiologic:* from bypass, to tissue reconstruction, to preservation and regeneration of lymphatic physiology. The dashed boundary distinguishes established clinical practice from technologies whose evidence base remains preliminary.

Source: Original figure prepared by the author.

CHAPTER 24

In Silico and Organ-on-a-Chip 3D Liver Analysis: Integrative Advances in Hepatic Modelling

Arooj Anwaar[1]
Derya Akyıldız Üstüner[2]
Mehmet Cengiz Üstüner[3]

[1] MSc., Eskişehir Osmangazi University, Department of Stem Cell Science, 0009-0006-7148-3961
[2] Assistant Professor, Eskişehir Osmangazi University, Vocational School of Health Services, Medical Laboratory Program, 0000-0002-8511-946X
[3] Professor, Eskişehir Osmangazi University, Basic Medical Sciences, Department of Medical Biology, 0000-0001-9802-3988

Introduction

The liver is an essential organ in pharmacology and toxicology because of its main function in the metabolism of endogenous and xenobiotic substances. Drug-induced liver injury (DILI) is a major contributor to post-marketing drug withdrawals and drug attrition during clinical trials, resulting in substantial financial losses and patient morbidity on a global scale (Kaur et al., 2023)

Regarding the early detection of hepatotoxic substances to prevent late-stage failures and guarantee patient safety, accurate liver models are essential (Karlsen et al., 2022).

Furthermore, liver illnesses with complex pathophysiologies involving numerous cell types and signaling pathways, such as cirrhosis, fibrosis, hepatitis, and nonalcoholic fatty liver disease (NAFLD), are significant worldwide health burdens. In order to comprehend disease mechanisms and create efficient treatments, it is essential to model these diseases in vitro. The intricate structure of the liver, which is made up of sinusoidal endothelial cells, hepatocytes, Kupffer cells, and hepatic stellate cells grouped in a highly ordered three-dimensional microenvironment, controls how the liver functions and reacts to damage. For translational relevance, models must capture this complexity (Nevzorova, Boyer-Diaz, Cubero, & Gracia-Sancho, 2020)

Liver models used in drug research must faithfully mimic metabolic pathways unique to humans, such as phase II conjugation, transporter functions, and cytochrome P450 enzyme activity (Polidoro, Ferrari, Marzorati, Lleo, & Rasponi, 2021). This is crucial because metabolic variations between species frequently lead to inaccurate human interpretations of animal data. Furthermore, more sophisticated systems that can replicate long-term liver function and immunological responses are required since chronic toxicity and idiosyncratic drug reactions are frequently not expected by traditional models.

Because they are easy to use and reasonably priced, traditional 2D monolayer cultures of hepatocytes or liver-derived cell lines have been employed extensively (Rezvani, Vallier, & Guillot, 2023). These models, however, rapidly lose liver-specific activities, such as reduced cytochrome P450 activity, changed gene expression, and a lack of physiological connections between cells and between cells and the matrix. These drawbacks restrict its applicability for long-term research and precise drug toxicity and metabolism prediction (Karlsen et al., 2022).

Preclinical liver research has relied heavily on animal models, especially rats. Their predictive ability for human liver reactions is diminished by notable interspecies variations in liver morphology, enzyme expression, immunological responses, and drug transporter profiles, notwithstanding their usefulness

(Lambrecht & Tacke, 2021). For instance, some medications that cause liver damage in humans do not cause liver damage in rats, and vice versa. Animal use is further limited by low throughput, high expenses, and ethical considerations. Due to the shortcomings of these conventional models, drug research has witnessed significant attrition rates, with many compounds failing because of unexpected hepatotoxicity or insufficient efficacy. This demonstrates the pressing need for more human-relevant, physiologically-relevant liver models that can faithfully replicate the mechanisms of liver disease and function (Nasiri-Ansari et al., 2022).

Two complementary advanced platforms—in silico computational modeling and organ-on-a-chip (OoC) technologies—have arisen to address the shortcomings of traditional models. These methods seek to enhance predictability, promote mechanistic understanding, and replicate the liver's intricate physiology and microenvironment.

In silico models use computer techniques to model drug metabolism, disease development, and liver physiology (Karlsen et al., 2022). These include multiscale simulations, agent-based models, physiologically based pharmacokinetic (PBPK) models, and, more and more, machine learning and artificial intelligence (AI) techniques. PBPK models allow for the prediction of drug behavior in individuals and specific populations by simulating drug absorption, distribution, metabolism, and excretion based on physiological factors and enzyme kinetics (Peiseler et al., 2022).

Multiscale and agent-based models provide mechanistic insights into liver disease and function by capturing tissue-level events and cellular interactions. Through the analysis of massive datasets and the discovery of intricate patterns in medication reactions and toxicity, artificial intelligence (AI) and machine learning algorithms improve predictive accuracy (Bruneau, Hundertmark, Guillot, & Tacke, 2021; Shroff et al., 2021).

Scalability, quick compound screening, affordability, and the capacity to combine various datasets are benefits of in silico models. Additionally, they facilitate the creation of hypotheses and virtual experiments, which direct the planning of in vitro and in vivo research (Takahashi et al., 2007). They must be integrated with experimental validation, nevertheless, because their correctness is dependent on the caliber of the input data and model assumptions.

Organ-on-a-chip technology recreates functioning organ units on microfabricated devices by combining tissue engineering, cell biology, and microfluidics. Multiple liver cell types are organized in three-dimensional (3D) structures within microfluidic channels that mimic mechanical forces and blood flow in liver-on-a-chip systems (Shroff et al., 2022).

Compared to conventional cultures, these systems have the following advantages:(Kaur et al., 2023)

• Physiological Microenvironment: Shear stress and nutritional gradients are provided by dynamic flow conditions, which enable the long-term preservation of liver-specific processes such protein synthesis, bile canaliculi production, and cytochrome P450 activity.

• Multicellular Complexity: Modeling immunological responses and cell-cell communication, which are essential for disease and toxicity research, is made possible by co-culturing hepatocytes with non-parenchymal cells (such as Kupffer cells and endothelial cells).

• Real-Time Monitoring: Biomarkers, metabolic activity, and cellular reactions can all be continuously measured thanks to integrated sensors

• High-Throughput Potential: Multiple chemicals or circumstances can be tested in simultaneously thanks to miniature platforms.

When compared to 2D cultures and animal models, these platforms have shown greater predictivity in hepatotoxicity screening, identifying medications that induce chronic toxicity and idiosyncratic liver injury. Additionally, they enable personalized treatment by modeling individual reactions utilizing patient-derived cells (Wang, Marucci, & Homer, 2025).

Purpose of this review

When compared to 2D cultures and animal models, these platforms have shown greater predictivity in hepatotoxicity screening, identifying medications that induce chronic toxicity and idiosyncratic liver injury. Additionally, they enable personalized treatment by modeling individual reactions utilizing patient-derived cells (McDevitt & Palecek, 2008).

The goal of this review is to present a thorough synthesis of current developments in 3D liver modeling, given the quick development of in silico and organ-on-a-chip technologies. It will specifically (Figeys & Pinto, 2000):

• Provide an overview of the most recent in silico and OoC liver models, emphasizing their design principles, uses, and efficacy in drug development and disease modeling (Ferry, Razinkov, & Hasty, 2011);

• Critically assess the advantages and disadvantages of each strategy, taking into account factors like scalability, physiological relevance, and predictive accuracy.

• Examine new approaches to combining computational and experimental platforms, such as virtual testing of OoC experiments and feedback loops for model improvement.

• Talk about the current issues with validation, standardization, and regulatory processes.

This review aims to educate researchers, physicians, and industry stakeholders on the transformative possibilities of merging organ-on-a-chip and in silico technologies by offering an integrative viewpoint. This confluence has the potential to speed up tailored therapy, enhance drug safety evaluation, and transform hepatic modeling (Takahashi et al., 2007).

Liver physiology and the need for advanced modeling

In mammalian physiology, the liver is a key organ that serves as a metabolic center that regulates a variety of vital processes. Protein synthesis, detoxification, and metabolism are some of its most important functions (Ghaffarizadeh, Heiland, Friedman, Mumenthaler, & Macklin, 2018). The liver is the primary location for amino acid catabolism, processes lipids and cholesterol, and controls glucose homeostasis through the storage of glycogen and gluconeogenesis (Sumadewi, 2023). Additionally, it uses the cytochrome P450 enzyme system to detoxify both endogenous and exogenous substances, such as medications and environmental pollutants. Furthermore, the liver produces and secretes bile acids required for lipid breakdown and absorption, as well as essential plasma proteins including albumin and clotting factors (Ozougwu, 2017).

In addition to these roles, the liver is essential for immunological modulation because it serves as a first line of defense against infections that enter the circulation from the gastrointestinal tract. It has a sizable population of resident immune cells, such as specialized macrophages called Kupffer cells, which provide tolerance and immunological surveillance (Trefts et al., 2019).

Architectural and cellular complexity of the liver

The liver's highly structured and diverse architecture supports its sophisticated functional design. The hepatic lobule, a roughly hexagonal arrangement of hepatocyte plates radiating from a central vein, is the fundamental structural unit of the liver (Qadri, Javaid, Reyaz, Ganie, & Reshi, 2025).The hepatic artery, portal vein, and bile duct branches that make up the portal triads encircle each lobule. Blood enters at the lobule's periphery via the nutrient-rich portal vein and oxygen-rich hepatic artery, then travels to the central vein via sinusoids, which are specialized capillaries. Metabolic zonation is the result of gradients of oxygen, nutrients, and hormones created throughout the lobule by this special dual blood supply (Trefts et al., 2019).

Within each lobule, hepatocytes which comprise roughly 60% of the liver's cellular mass—are arranged in concentric layers. Near the portal triads, the periportal (zone 1) hepatocytes are subjected to elevated oxygen and nutrient concentrations and are principally engaged in urea production, gluconeogenesis,

and oxidative metabolism (Sumadewi, 2023). On the other hand, pericentral (zone 3) hepatocytes, which are located close to the central vein, operate in more hypoxic environments and are focused on glutamine synthesis, glycolysis, and xenobiotic metabolism. The characteristics of the mid-lobular (zone 2) cells are intermediate.

The existence of non-parenchymal cells complicates this spatial compartmentalization even more including:

• Sinusoidal endothelial cells: These cells line the sinusoids and help hepatocytes and blood exchange.

• Kupffer cells: Local macrophages that aid in debris removal and immunological protection.

• Hepatic stellate cells: Control the synthesis of extracellular matrix and store vitamin A.

•Cholangiocytes: These cells line the bile ducts and aid in the secretion and alteration of bile (Mohajan, 2025).

The liver's dynamic microenvironment and the interaction of these many cell types allow for the simultaneous execution of opposing metabolic activities and quick adaptability to physiological demands. The architecture of the liver also facilitates the development of the bile canalicular network, which exhibits structural and functional heterogeneity of its own. Hepatocyte-produced bile flows from the lobule's core toward its periphery in the opposite direction of blood (Dalsbecker, Beck Adiels, & Goksör, 2022).

Requirement for functional mimicry in models

Accurately simulating the liver's function in vitro or in silico is extremely difficult due to its complexity. The multicellular structure, metabolic zonation, and dynamic milieu of the human liver are frequently not replicated by conventional two-dimensional (2D) cell cultures and animal models, which limits their ability to predict drug metabolism, toxicity, and the course of disease (Hassan et al., 2020)

Advanced models must take into account a number of crucial factors in order to accomplish functionalmimicry:

• Reproduction of Metabolic Zonation: The geographical gradients of hormones, nutrients, and oxygen that influence the functional heterogeneity of hepatocytes should be represented in models. This is essential for accurately modeling lobule-differentially localized activities such drug biotransformation, lipid metabolism, and ammonia detoxification (Soldatow, LeCluyse, Griffith, & Rusyn, 2013).

Replicating the entire range of liver functions, such as immune responses, matrix remodeling, and bile secretion, requires the inclusion of non-parenchymal cells (endothelial cells, Kupffer cells, stellate cells, and cholangiocytes) in addition to hepatocytes (Bedossa, 2017).

• 3D Architecture and Microenvironment: To allow for appropriate cell-cell and cell-matrix interactions, three-dimensional (3D) models must replicate the lobular structure, sinusoidal network, and bile canaliculi. For instance, controlled perfusion, shear stress, and the creation of physiological gradients are made possible by microfluidic organ-on-a-chip platforms, which provide cellular activity that is more akin to that of cells in vivo (van Grunsven, 2017).

• Dynamic and Real-Time Monitoring: To offer dynamic insights into liver physiology and disease, advanced systems should allow for real-time evaluation of liver function, including metabolic activity, enzyme induction, and biomarker production.

• Scalability and Reproducibility: Liver models need to be scalable, repeatable across labs, and able to withstand high-throughput screening in order to be widely used in drug discovery and disease modeling (Pingitore et al., 2019).

The case for advanced modeling

These issues have started to be addressed by recent developments in in silico modeling and liver-on-a-chip technology. Liver-on-a-chip technologies replicate elements of the hepatic milieu, including perfusion, oxygen gradients, and three-dimensional cell structure, by integrating several cell types within a microfluidic platform. Compared to static 2D cultures, these platforms have shown better maintenance of liver-specific activities, such as cytochrome P450 activity, albumin synthesis, and bile canaliculi formation (Manmadhan & Ehmer, 2019).

To forecast hepatic drug metabolism and toxicity, in silico models—such as physiologically based pharmacokinetic (PBPK) models and agent-based simulations—can integrate comprehensive anatomical, cellular, and molecular data. These computational methods allow for virtual experimentation and iterative model improvement, which further improves the prediction ability of liver models when paired with experimental data from liver-on-a-chip systems (Zhang et al., 2017).

The liver's complex architecture and cellular heterogeneity are closely related to its distinct set of metabolic, synthetic, and immunological functions. To capture this complexity and produce physiologically meaningful predictions of liver function, drug response, and disease progression, sophisticated modeling techniques—both computational and experimental—are necessary (Deguchi & Takayama, 2022). The combination of liver-on-a-chip and in silico models has great potential to close the gap between conventional in vitro systems and the

human liver in vivo, which will ultimately advance drug development and personalized medicine as technologies continue to advance.

In silico models for the liver

Because of its crucial function in metabolism, detoxification, and homeostasis, the liver is a focus of sophisticated in silico modeling. Drug research, toxicity prediction, and disease modeling can all benefit greatly from the simulation and prediction of hepatic processes made possible by these computational methods (Soldatow et al., 2013). With a focus on their latest developments and applications, this section examines the three main types of in silico liver models: physiologically based pharmacokinetic (PBPK) models, agent-based and multiscale models, and machine learning/artificial intelligence (AI) techniques (Moravcová et al., 2015).

Physiologically based pharmokinetic PBPK Models

PBPK models are mechanistic mathematical frameworks that depict the body as interconnected compartments, each of which corresponds to an organ or tissue, including the liver, in order to mimic the absorption, distribution, metabolism, and excretion (ADME) of substances. By combining biochemical processes (such enzyme kinetics) with physiological characteristics (like organ volumes and blood flow rates), these models allow for quantitative estimates of drug concentrations over time in plasma and particular organs (Ashmore-Harris, Antonopoulou, Finney, et al., 2024).

Important characteristics and uses include:

• Mechanic Representation: By including liver-specific data including hepatic blood flow, enzyme activity, and transporter expression, PBPK models explicitly mimic hepatic drug metabolism. This makes it possible to accurately forecast the levels of drugs and metabolites in the liver and bloodstream.

• Drug Development: To forecast liver exposure to medications and biologics, guide dose selection, and evaluate the risk of hepatotoxicity, the pharmaceutical sector frequently uses PBPK models. They are endorsed by organizations like the FDA and EMA and are being utilized more frequently in regulatory submissions.

• Personalized Medicine: PBPK models can offer tailored estimates of drug kinetics and reactions by incorporating patient-specific data (such as genetic polymorphisms, illness states) (Campbell et al., 2026).

Agent based and multiscale models

From the cellular to the tissue and organ levels, the spatial and temporal complexity of the liver is captured by agent-based models (ABMs) and multiscale models. ABMs allow for the simulation of emergent phenomena like tissue

regeneration, fibrosis, and metabolic zonation by modeling individual things (cells, molecules) as "agents" with distinct behaviors and interactions (Brennan et al., 2021).

Important characteristics and uses include: • Spatial and Temporal Resolution: ABMs can replicate the complex cellular heterogeneity, microenvironmental gradients, and liver lobule architecture, offering insights into how local interactions influence liver function globally.

• Disease Modeling: Research on liver regeneration, fibrosis, steatosis, and the development of acute and chronic liver illnesses has been conducted using these models.

• Integration Across Scales: Multiscale models provide a comprehensive understanding of liver physiology and pathology by connecting molecular signaling pathways to tissue- and organ-level processes.

Organ on a chip liver models

Microfluidics and liver on a chip systems

Liver modeling has been transformed by microfluidic organ-on-a-chip (OOC) technology, which makes it possible to recreate physiologically relevant microenvironments such as dynamic flow, shear stress, and three-dimensional multicellular architecture. Liver-on-a-chip (LOC) devices enable for regulated perfusion and nutrient delivery by simulating the hepatic sinusoidal network using microfabricated channels. Important elements of the hepatic microenvironment, such as oxygen and nutrient gradients, which are essential for preserving hepatocyte viability and function, can be replicated by these systems (Ashmore-Harris, Antonopoulou, Aird, et al., 2024).

Integrated gut-liver-on-a-chip (GLOC) models, which mimic the gut-liver axis and enable the investigation of inter-organ communication, metabolic processing, and immunological interactions, have been made possible by recent developments in microfluidic engineering. Materials like as polydimethylsiloxane (PDMS), polymethyl methacrylate (PMMA), and polycarbonate (PC) are used to construct OC devices; each has unique benefits in terms of scalability, optical clarity, and biocompatibility (Alfaifi, Eom, Newsome, & Baik, 2018).

The creation of physiologically significant shear forces and concentration gradients is made possible by the exact control of fluid dynamics in microfluidic devices. Because hepatocytes are extremely sensitive to mechanical and biochemical inputs, this is crucial for simulating the liver environment in vivo. To increase their usefulness for drug screening and disease modeling, LOC

systems frequently include embedded sensors for real-time monitoring of oxygen, metabolites, and other functional indicators (Farhang Doost & Srivastava, 2024).

Biomaterials and 3D scaffolds

In order to replicate the extracellular matrix (ECM) and promote the development of functional liver tissue in LOC systems, the choice of biomaterials and the creation of three-dimensional scaffolds are essential. Because of its simplicity of microfabrication, gas permeability, and biocompatibility, PDMS continues to be the most extensively utilized polymer. However, because to their better mechanical qualities and mass production compatibility, substitute materials like PMMA, PS, and PC are becoming more and more popular (Whitesides, 2006)

The 3D scaffolds used in LOC devices are made of either synthetic or natural polymers, such as collagen, gelatin, and Matrigel, and they offer a supportive environment for hepatocyte attachment, proliferation, and differentiation. Compared to conventional 2D culturing, these scaffolds help generate hepatocyte spheroids or organoids, which more accurately replicate the structure and function of the liver in vivo (Ingber, 2022).

Decellularized liver extracellular matrix (ECM) has been shown in recent research to be used as a scaffold, providing a native-like microenvironment that improves hepatocyte lifetime and function. The precise spatial organization of various cell types and ECM components within LOC platforms is made possible by the incorporation of 3D bioprinting technology, which has further improved the field (Wu et al., 2020).

Co cultures and vascularization strategies

LOC models frequently use co-culture techniques incorporating hepatocytes and non-parenchymal cells, such as hepatic stellate cells, Kupffer cells, and liver sinusoidal endothelial cells (LSECs), in order to faithfully replicate liver physiology. The study of intricate intercellular relationships, immunological responses, and pathological processes like fibrosis is made possible by these multicellular systems (Wu et al., 2020).

The preservation of tissue viability and function depends on vascularization. Advanced LOC platforms use endothelial cells to create microvascular networks that facilitate waste disposal and the delivery of nutrients and oxygen. Certain designs imitate the hepatic sinusoidal network by using hollow fiber membranes or microfabricated channels, which encourages the development of continuous microvascular structures that sustain liver tissue's long-term culture and functional maintenance (Figeys & Pinto, 2000).

Evaluation of function; biomarkers, imaging, real time monitoring

For LOC models to be used in industry and research, functional validation is necessary.

Among the important evaluation metrics are, Morphological Assessment: Histological staining (such as H&E) and immunofluorescence to confirm cell identification and tissue architecture, Biomarker Expression: Identification of endothelium (like VE-cadherin), hepatocyte (like CYP3A4, albumin), and stellate cell (like vimentin) markers, Drug Metabolism: Enzyme activity assays (such as cytochrome P450 activity) to evaluate metabolic capacity; Secretory Function: Measurement of albumin secretion and urea synthesis as markers of hepatic function; and Real-Time Monitoring: Combining biosensors and imaging modalities to continuously monitor cellular responses, metabolite production, and toxicity events (Tehranirokh, Kouzani, Francis, & Kanwar, 2013).

Integration of in silico and organ on a chip approaches

Controlling Microfluidic Systems Computationally realtime computational control of microfluidic parameters is made possible by the combination of LOC technology with in silico modeling. In order to guide the design and optimization of experimental conditions, computational fluid dynamics (CFD) models can forecast flow patterns, shear stress, and nutritional gradients within LOC devices. Algorithms can modify dosing schedules and flow rates in response to real-time input from embedded sensors, increasing experimental throughput and reproducibility (Miri et al., 2019).

Computational control of microfluidic systems

Real-time computational control of microfluidic parameters is made possible by the combination of LOC technology with in silico modeling. In order to guide the design and optimization of experimental conditions, computational fluid dynamics (CFD) models can forecast flow patterns, shear stress, and nutritional gradients within LOC devices. Algorithms can improve experimental throughput and reproducibility by modifying flow rates and dosing regimens in response to real-time feedback from embedded sensors (Ingber, 2022).

Feedback loops; data driven model refinement

A feedback loop that improves predicted accuracy can be created by using data from LOC trials to improve in silico models. Large datasets from LOC platforms are analyzed by machine learning and artificial intelligence algorithms to find trends, optimize model parameters, and raise the accuracy of computer simulations. New therapeutic targets and drug candidates are found more quickly because to this iterative procedure, which also facilitates hypothesis testing (Chen et al., 2022).

Virtual testing of OoC experiments and vice versa

In silico models eliminate the need for expensive and time-consuming physical experiments by enabling the virtual testing of experimental circumstances. On LOC systems, virtual screening can prioritize conditions for validation, forecast results, and determine ideal settings. On the other hand, information from LOC trials ensures that computational models are relevant to human physiology by validating and calibrating them (Chen et al., 2022; Clevers, 2016).

Case studies of combined systems

Recent research has shown that in silico and LOC techniques can be successfully combined. For instance, LOC devices that mimic physiological shear stress and nutritional gradients have been designed using CFD simulations, and the models' accuracy has been confirmed experimentally6. Our knowledge of the gut-liver axis has advanced thanks to integrated gut-on-a-chip models and computer simulations that have shed light on immune responses, metabolite processing, and nutrition transfer (Liu et al., 2025).

Current challenges and limitations

Standardization and reproducibility

The absence of standardized definitions, cell sources, structural designs, and validation procedures for LOC models is a significant obstacle in the field. Reproducibility and comparability may be limited by variable results resulting from variations in chip manufacture, cell source, and experimental settings. These problems are being addressed by recent initiatives, such as the establishment of national and international standards for liver-on-a-chip technology, which include thorough instructions for model building, quality assurance, and functional validation (Tao et al., 2022).

Scalability and regulatory acceptance

Although LOC platforms have several advantages over conventional models, they are still not widely used in industry and regulations due to their restricted scalability for high-throughput applications. The integration of many cell types, the complexity of device production, and the requirement for automated methods to facilitate large-scale research are among the difficulties. Strong validation and proof of the model's applicability to human physiology are necessary for regulatory approval of LOC data for medication development and safety evaluation (Liu et al., 2025; Tao et al., 2022).

Data integration and model validation

Standardized data formats and interoperable platforms are necessary for the complicated task of integrating data from various sources, like as clinical research, LOC experiments, and in silico models. To guarantee that forecasts and experimental results are reliable, model validation is essential. This entails setting precise standards for functional performance and predicting accuracy as well as comparing LOC and computational models to gold-standard data, such as primary human tissue or clinical outcomes (Beckwitt et al., 2018).

AI-Enhanced modeling and real time biofeedback

LOC systems' incorporation of machine learning and artificial intelligence (AI) has the potential to completely transform the industry. Artificial intelligence (AI) algorithms are able to predict cellular reactions, evaluate real-time data from embedded sensors, and dynamically modify experimental circumstances to maximize results. Personalized modeling, adaptive testing, and quicker identification of illness processes and treatment options are all made possible by this method (Esch, Bahinski, & Huh, 2015; Kaur et al., 2023).

Future prospects

Personalized liver on a chip systems

Organoid culture and stem cell technological advancements have made it possible to create customized LOC models with patient-derived cells. Precision medicine initiatives can be supported by these systems, which are able to capture individual diversity in medication metabolism, illness susceptibility, and treatment response. By facilitating customized testing and modeling, personalized LOC platforms offer the potential to enhance medication safety, efficacy, and patient outcomes.

Integration into multiorgan systems

The creation of "human-on-a-chip," or multi-organ-on-a-chip, systems is a significant advancement in the field. These platforms simulate systemic interactions and pharmacokinetics by integrating numerous organ models, including the gut, liver, kidney, and heart, and connecting them via microfluidic channels. A more thorough understanding of human biology and disease is made possible by multi-organ systems, which allow the study of intricate physiological processes, inter-organ communication, and multi-organ toxicity.

Path to clinical translation ad regulatory frameworks

Strong validation, standardization, and proof of therapeutic relevance are necessary for LOC technology to be widely adopted in clinical and regulatory settings. Establishing standards for model building, data reporting, and

performance benchmarking requires cooperation between academic institutions, business, and government organizations. Clinical adoption of these technologies will be accelerated by the creation of regulatory systems that accept LOC data for drug approval and safety evaluation.

Conclusion

Hepatic modeling has been revolutionized by organ-on-a-chip technology, which makes it possible to recreate physiologically realistic liver microenvironments and supports applications in toxicology, drug development, and disease modeling. Complex LOC systems that closely resemble the structure and function of the human liver have been created thanks to developments in microfluidics, biomaterials, 3D scaffolding, and co-culture techniques. LOC systems' prediction capabilities have been further improved by the use of in silico modeling, facilitating data-driven discovery and personalized therapy.

There are still issues with standardization, scalability, and regulatory approval despite tremendous advancements. For LOC technology to continue to grow and be adopted, it is imperative that strong validation methods, defined rules, and interoperable data platforms be developed. With AI-enhanced, customized, multi-organ systems poised to transform drug development, toxicity, and clinical care, the future of hepatic modeling lies at the intersection of biology and computer.

In the end, combining in silico and organ-on-a-chip techniques could result in more precise, effective, and human-relevant models for pharmacology, toxicology, and personalized medicine, opening the door to safer and more successful treatments.

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