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CONTENTS

CHAPTER 1	7
Extracellular Vesicles and microRNAs in Cardiovascular Diseases	
Gokce Topal & Seynur Sunar	
CHAPTER 2	23
New Horizons In Food And Health: Exopolysaccharides Of Lactic Acid Bacteria	
Osman Eren	
CHAPTER 2	45
Usage of Advanced Machine Learning Tools for Treatment Optimization Within the Framework of Personalized Medication Management	
Hunaida Avvad	
CHAPTER 4	79
Antifungal Resistance and Emerging Therapeutic Strategies in Oral Candidiasis	
Özge Özdal Zincir	
CHAPTER 5	107
Guided Bone Regeneration in Oral Surgery: Biomaterials, Biological Principles, and Clinical Outcomes	
Özge Özdal Zincir	
CHAPTER 6	131
Biophysical Effects of TENS and NMES Applications	
Nüha Zehra Kılınç & Nurten Bahtiyar & Birsen Aydemir	
CHAPTER 7	149
Diagnosis and Clinical Management of Acute Diverticulitis	
Tarık Sırça	
CHAPTER 8	159
Clinical Approach to Thyroid Nodules	
Alper Çalışkan	

CHAPTER 9	167
Intraoral Maxillary Molar Distalization Methods in Class II Malocclusion Treatment	
Büşra Nur Arslan	
CHAPTER 10.....	191
Social Determinants of Periodontal Health: Implications for Health Equity and Clinical Practice	
Ilknur Sidika Tayfun Buyukeken & Sefa Aydınođan	
CHAPTER 11.....	205
Shaping The Future of Pathology: Use of Artificial Intelligence as a Component of Telepathology and Digital Pathology	
Behice Hande Erenler & Ali Kemal Erenler	
CHAPTER 12.....	217
Micrnas in the Pathogenesis of Sepsis-Associated Disseminated Intravascular Coagulation	
Gölfem Erbil	
CHAPTER 13.....	227
Modern Perspectives on Next-Generation Antioxidants in Signaling Pathway Regulation	
Mahira Firudin kizi Amirova	
CHAPTER 14.....	263
Inhibition of Cancer-Related Enzymes with Heavy Metals	
Cemile Zontul & Tugba Agbektas	
CHAPTER 15.....	275
Physiological Foundations and Systemic Implications of Microbiota–Circadian Rhythm Interactions	
Ali Karadeniz & Aysun Aksoy Yılmaz	
CHAPTER 16.....	299
Use of Social Media in Public Health: Vaccination, Vaccine Hesitancy and Digital Infodemic	
Serap Salimođlu	

CHAPTER 17 317
Complications Following Pancreatic Surgery: Prevention and Management
 Serhan Akalın

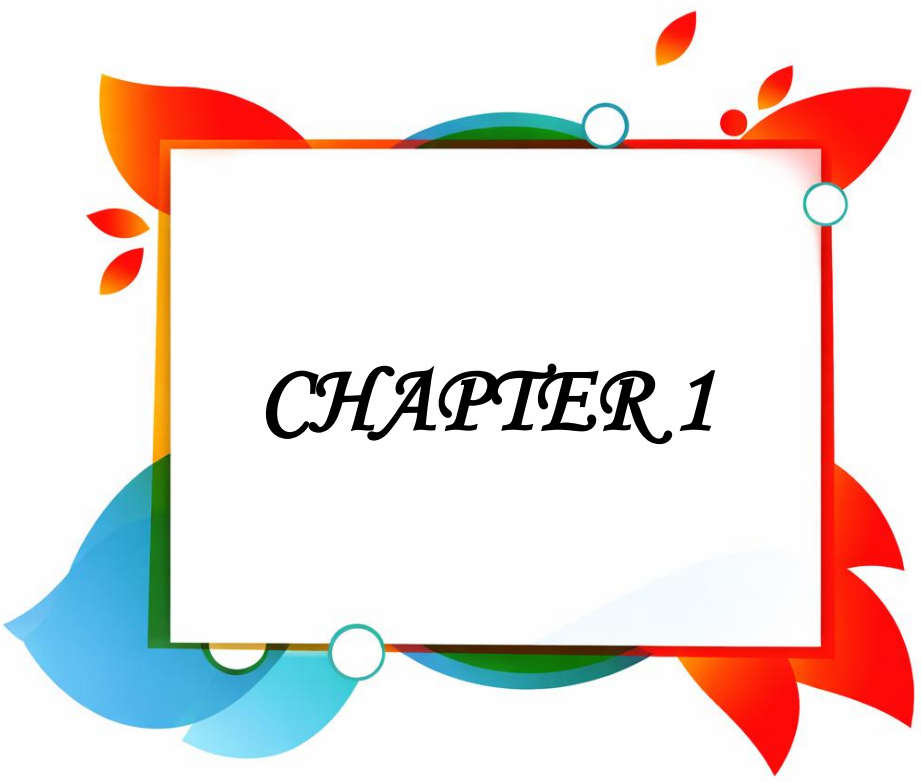
CHAPTER 18 335
Prosthetic Approaches in Pediatric Dentistry
 Büşra Dinç & Ayşenur Germeğenlioğlu & Şeref Tunay Kocabay

CHAPTER 19 383
Tooth Autotransplantation in Contemporary Dentistry: Biological Principles, Multidisciplinary Challenges, and Future Perspective
 Mustafa Üstün

CHAPTER 20 399
Histopathological Spectrum of Celiac Disease
 Seda Tas Ayçiçek

CHAPTER 21 409
Biomechanics of The Circulatory System
 Furkan Kayalar & İsmail Uysal & Bircan Yücekaya

CHAPTER 22 417
Molecular Epidemiology and Regional Distribution of *Echinococcus Granulosus* Genotypes in Türkiye: A Review of Human and Animal Isolates
 Saliha Zeynep Aydın

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CHAPTER 1

Extracellular Vesicles and microRNAs in Cardiovascular Diseases

Gokce Topal¹ & Seynur Sunar²

1. Introduction

Cardiovascular diseases remain the leading cause of morbidity and mortality worldwide (Botts et al., 2021). Among these diseases, atherosclerosis plays a central role in the development of many cardiovascular complications such as myocardial infarction and stroke (Botts et al., 2021). The pathogenesis of cardiovascular diseases involves complex interactions between vascular cells, circulating immune cells, and molecular signaling pathways (Howe et al., 2022).

Recent findings demonstrated that intercellular communication is not only mediated by classical signaling molecules such as cytokines and growth factors, but also by extracellular vesicles (van Niel et al., 2018). Extracellular vesicles are small membrane-bound structures released by cells into the extracellular environment and play a crucial role in cell-to-cell communication (van Niel et al., 2018).

Extracellular vesicles carry a wide range of biologically active molecules, including proteins, lipids, DNA, messenger RNA (mRNA), and microRNAs (miRNAs) (van Niel et al., 2018). Through the transfer of these molecules, extracellular vesicles can influence the phenotype and function of recipient cells (Botts et al., 2021). Recent research has highlighted the importance of extracellular vesicle-mediated miRNA transport in cardiovascular diseases (Botts et al., 2021).

2. Extracellular Vesicles

Extracellular vesicles are membrane vesicles secreted from various cells (van Niel et al., 2018). The secretion of extracellular vesicles was initially described as a disposal system for unneeded compounds in the cell (Johnstone et al., 1987).

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Recent findings revealed that extracellular vesicles play an important role in intercellular communication and involved in diverse biological processes (van Niel et al., 2018). These vesicles are divided into exosomes (also known as small extracellular vesicles), microvesicles and apoptotic bodies based on their size and biogenesis (Théry et al., 2018).

2.1. Exosomes

Exosomes are small extracellular vesicles (50-200 nm in diameter), first described in reticulocytes during differentiation (Johnstone et al., 1987). These vesicles originate from endosomal system, and secreted upon fusion of multivesicular bodies (MVB) with the plasma membrane (van Niel et al., 2018).

2.2. Microvesicles

Microvesicles, formerly called 'platelet dust', were first described originating from platelets in normal plasma and serum (Wolf, 1967). Microvesicles range in size from 50 nm to 1 μ m in diameter. They are generated by the outward budding of the plasma membrane and secreted into the extracellular space (van Niel et al., 2018). These vesicles can also reach up to 10 μ m in cancer cells, called as 'oncosomes' (Al-Nedawi et al., 2008).

2.3. Apoptotic bodies

Apoptotic bodies are extracellular vesicles, generated by apoptotic cells. These vesicles are produced by the plasma membrane blebbing during apoptosis (van Niel et al, 2018). Apoptotic bodies range between 1 μ m to 5 μ m in diameter (van Niel et al, 2018).

3. Biogenesis of Extracellular Vesicles

Multiple molecules have been identified to play a role in the biogenesis of exosomes, including endosomal sorting complex required for transport (ESCRT) (Hurley, 2008). This machinery consists of four subunits (ESCRT-0, -I, -II, -III) that act in a stepwise manner. Exosomes can also be formed in an ESCRT-independent manner (Stuffers et al., 2009). The first ESCRT-independent mechanism of exosome biogenesis was shown to require generation of ceramide by neutral type II sphingomyelinase, which hydrolyses sphingomyelin to ceramide (Trajkovic et al., 2008). Also, proteins of the tetraspanin family have been shown to regulate ESCRT-independent endosomal sorting (van Niel et al., 2011). The activity of the Rho family of small GTPases and of the Rho-associated protein kinase (ROCK) has shown to play a role in microvesicle biogenesis in cancer cells (Li et al., 2012).

Once released into the extracellular space, extracellular vesicles can be taken

up by recipient cells. After uptake, they can undergo endocytosis and reach multivesicular endosomes (MVE), which, in most cases, are targeted to the lysosome (Tian et al., 2010). Extracellular vesicles can also elicit functional responses by binding to and activating receptors expressed on the recipient cells (Raposo et al., 1996). Alternatively, they can fuse directly with the plasma membrane or endocytic membrane of recipient cells, which requires the release of the content from extracellular vesicles into recipient cells (Valadi et al., 2007).

4. Molecular Cargo of Extracellular Vesicles

Extracellular vesicles can deliver their contents to elicit functional responses in the recipient cell (Zaborowski et al., 2015). The content of the extracellular vesicle is influenced by the physiological or pathological state of the secreting cell (van Niel et al, 2018). Extracellular vesicle cargo can contain proteins, lipids, and nucleic acids such as mRNA and miRNA (van Niel et al, 2018). Among these, miRNAs have attracted particular attention due to their ability to regulate gene expression.

4.1. MiRNA transport by Extracellular Vesicles

MiRNAs are small (21-23 nucleotides), non-coding RNAs that regulate expression (Tymoczko et al., 2011). Binding of the miRNA to its complementary mRNA target results in inhibition of translation or mRNA degradation (Alberts et al., 2014). MiRNAs have been shown to regulate a wide variety of biochemical processes including development, cell differentiation, and oncogenesis (Tymoczko et al., 2011).

MiRNAs, transferred in extracellular vesicles, can affect signaling and gene expression in recipient cells (Fig. 1A). For example, the anti-inflammatory miRNA-10a was transferred to monocytic cells by quiescent endothelial cell-derived extracellular vesicles, and repressed inflammation by targeting components of the nuclear factor-kappa B (NF- κ B) inflammatory pathway (Njock et al., 2015). Extracellular vesicle-derived miRNA communication has been shown to play an important role in the regulation of various biological processes including immunity, inflammatory signaling, cancer, and vascular functions (Kalluri & LeBleu, 2020).

5. Extracellular Vesicles in Cardiovascular System

Within the cardiovascular system, extracellular vesicles are secreted by a wide range of cell types, including endothelial cells, vascular smooth muscle cells, cardiomyocytes, platelets, and immune cells (Fu et al., 2020). These vesicles regulate processes such as inflammation, thrombosis and angiogenesis (Coly & Boulanger, 2022). Through the transfer of molecular cargo, extracellular vesicles

participate in the regulation of vascular homeostasis as well as the development of cardiovascular diseases (Coly & Boulanger, 2022).

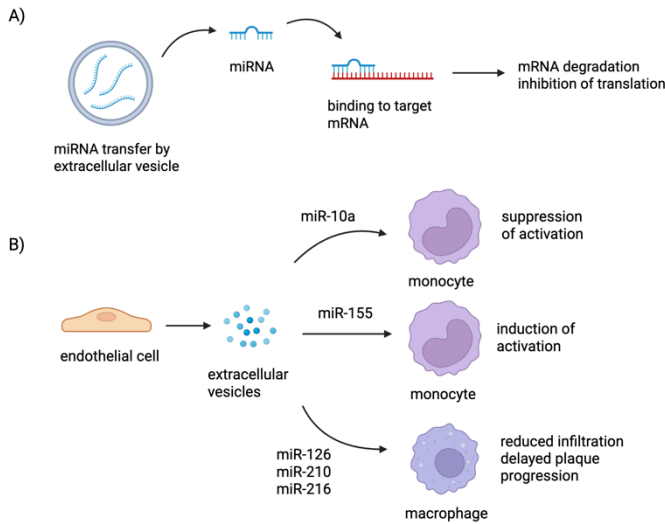


Figure 1. A) miRNAs, transferred by extracellular vesicles, bind to target mRNAs and can regulate gene expression in recipient cells. B) Endothelial cell-derived extracellular vesicles can regulate cells involved in atherosclerosis via miRNAs.

5.1. Role of Extracellular Vesicles in Atherosclerosis

Atherosclerosis is a chronic inflammatory disease characterized by lipid accumulation and immune cell infiltration within the arterial wall (Howe et al, 2022). In recent years, vascular cell-cell communication mediated by the secretion of extracellular vesicles has attracted much research interest for the pathogenesis and treatment of atherosclerosis (Botts et al., 2021). Extracellular vesicles have been shown to modulate vascular function in atherogenesis via intercellular signaling between endothelial cells (ECs) and other cell types (Botts et al., 2021).

Extracellular vesicle-derived apoptotic bodies containing miR-126 were found to reduce macrophage content in plaque (Zernecke et al., 2009). EC-extracellular vesicles have been demonstrated to regulate both immune and non-immune cells involved in the development of atherosclerosis (Li M. et al., 2018; He et al., 2018) (Fig. 1B). Exosomal miRNAs have been shown to mediate crosstalk between ECs and macrophages by suppressing (e.g., miR-10a) (Njock et al., 2015) or promoting monocyte activation (e.g., miR-155) (He et al., 2018), reducing macrophage infiltration, and delaying plaque progression (e.g., miR-126, miR-210, and miR-216) (Wang et al., 2019b).

Monocyte- and macrophage-derived extracellular vesicles have been demonstrated to induce EC apoptosis and promote murine atherosclerosis through elevated blood lipids, oxidative stress, and inflammation (Aharon et al., 2008), as well as by reducing proliferation and angiogenesis in human coronary artery ECs via miR-503-5p (Wang et al., 2021).

Communication via extracellular vesicles between cells of the vasculature has been observed in ECs and smooth muscle cells (SMCs) as well, where EC secreted extracellular vesicles induce both atheroprone (Boyer et al., 2020; Yuan X. et al., 2020; Zhang Z. et al., 2020) and atheroprotective SMC phenotypes (Hergenreider et al., 2012), while SMC-derived extracellular vesicles govern endothelial migration under stimulation by platelet derived growth factor (Heo et al., 2020).

6. Isolation and Characterization of Extracellular Vesicles

A variety of methods have been developed for the isolation and characterization of extracellular vesicles.

6.1. Isolation Methods

6.1.1. Differential Ultracentrifugation

Differential ultracentrifugation is based on the sequential removal of particles based on their size through different centrifugal durations and forces (Hadizadeh et al., 2022). The principle is to apply increasing centrifugal forces to the extracellular vesicle containing sample to pellet smaller particles gradually (Welsh et al., 2024). This method is considered as the gold standard technique for exosome isolation (Li et al., 2017). Differential ultracentrifugation separates cell debris, apoptotic bodies and microvesicles from the extracellular vesicle containing sample (Proestler et al., 2023), and is particularly well-suited for isolating extracellular vesicles from larger volumes, such as cell culture supernatant (Wang et al., 2024).

6.1.2. Density Gradient Ultracentrifugation

Density gradient ultracentrifugation combines ultracentrifugation with a gradient medium, typically sucrose or iodixanol, to distinguish extracellular vesicles from non-vesicular particles (Yu et al., 2018). Density gradient is selected for its ability to separate extracellular vesicles based on density. This method is particularly useful for studies requiring high purity extracellular vesicles (Wang et al., 2024).

6.1.3. Ultrafiltration

Ultrafiltration method is based on molecular size separation (Wang et al.,

2024). In this method, particles are separated based on pore size. In this process, larger molecules are retained while smaller ones are allowed to pass through. This method effectively concentrates extracellular vesicles from large volumes (Lobb et al., 2015).

6.1.4. Size Exclusion Chromatography

Size exclusion chromatography is a method for separation of particles based on size or molecular weight. The mechanism is based on the porous polymer microspheres packed into the chromatographic column. Through this column, smaller particles migrate more slowly than larger ones (Yang et al, 2020).

6.1.5. Polymer Precipitation

Polymer precipitation, originally developed for purifying viral particles, has been adapted for extracellular vesicles due to their shared physicochemical properties (Wang et al., 2024). This technique predominantly uses PEG for precipitation. PEG, a hydrophilic compound, attracts water molecules, leading to reduced solubility and aggregation of extracellular vesicles, facilitating their precipitation upon centrifugation (Soares Martins et al., 2018).

6.1.6. Anion Exchange Chromatography

Anion Exchange Chromatography (AEC) is a method, in which the negative charge of extracellular vesicles interact with charged resins within the chromatographic column (Heath et al., 2018; Koch et al., 2024).

6.1.7. Affinity-based Exosome Isolation

Affinity-based methods are based on specific biochemical interactions such as antigen-antibody reactions. These methods commonly use antibodies for separation (Neumair et al., 2023).

6.2. Characterization Methods

6.2.1. Quantification of Particle Size

Extracellular vesicles can be characterized by determination of particle size by methods such as Nanoparticle tracking analysis (NTA), and Dynamic Light Scattering (DLS) (Welsh et al., 2024).

6.2.2. Quantification of Total Protein

Total protein in an extracellular vesicle preparation can be determined by colorimetric assays, fluorometric assays, global protein stain on SDS-PAGE, or absorbance readings (Welsh et al., 2024).

6.2.3. Characterization of Extracellular Vesicles by Morphology

Extracellular vesicle morphology is currently assessed for smaller extracellular vesicles using electron microscopy such as: scanning electron microscopy (SEM) (Cavallaro et al., 2021) and transmission electron microscopy (TEM) (Corona et al., 2023).

6.2.4. Characterization of Extracellular Vesicles by Protein Composition

6.2.4.1. ExoView Analysis

The ExoView platform enables the detection of vesicle surface proteins and the characterization of specific extracellular vesicle subpopulations (Breitwieser et al., 2022).

7. Clinical and Therapeutical Potential

Extracellular vesicles have emerged as promising tools in cardiovascular medicine due to their role in intercellular communication and their ability to transport biologically active molecules, including proteins, lipids, and nucleic acids such as microRNAs (miRNAs). Increasing evidence suggests that extracellular vesicles contribute to both physiological and pathological processes in the cardiovascular system, highlighting their potential clinical utility (Botts et al., 2021).

Extracellular vesicle-associated miRNAs represent promising biomarkers because they are protected from degradation by the lipid bilayer of vesicles, making them stable in circulating body fluids such as plasma and serum. Changes in the expression patterns of extracellular vesicle-derived miRNAs have been associated with various cardiovascular conditions, including atherosclerosis (Howe et al., 2022). Consequently, profiling extracellular vesicle-associated miRNAs may enable the detection of cardiovascular diseases at early stages and support more precise patient stratification.

Beyond their diagnostic potential, extracellular vesicles are also being investigated as therapeutic strategies (Botts et al., 2021). Engineered extracellular vesicles can be used to deliver RNA-based therapeutics—including miRNA mimics, small interfering RNAs (siRNAs), or antisense oligonucleotides—as well as other bioactive molecules directly to target tissues. Extracellular vesicles exhibit low immunogenicity and favorable biocompatibility compared with many synthetic delivery systems. These properties make them potential candidates for targeted drug delivery aimed at modulating pathological pathways involved in cardiovascular disease.

Although the therapeutic application of extracellular vesicles remains largely at the experimental stage, ongoing advances can accelerate their translation into clinical practice. Collectively, extracellular vesicle-associated miRNAs represent a promising frontier in both the diagnosis and treatment of cardiovascular diseases.

8. Future Perspectives

Despite significant advances in recent years, many aspects of extracellular vesicle biology remain incompletely understood. Further research is necessary to clarify their physiological functions, molecular composition, and contributions to cardiovascular disease pathogenesis. Addressing these knowledge gaps will be essential for translating extracellular vesicle-related research into clinical practice.

A deeper understanding of the molecular mechanisms underlying extracellular vesicle biogenesis, cargo selection, and release is needed. Elucidating these processes will help clarify how specific miRNAs and other bioactive molecules are selectively packaged into extracellular vesicles and transferred to recipient cells within the cardiovascular system.

The development of standardized and reproducible extracellular vesicle isolation and characterization protocols is needed. Currently, variability in isolation techniques—including ultracentrifugation, precipitation-based methods, and size-exclusion chromatography—can significantly influence the composition and purity of extracellular vesicle preparations. Standardization will improve comparability between studies and facilitate the clinical validation of extracellular vesicle-derived biomarkers.

Another important direction involves the identification and validation of disease-specific extracellular vesicle biomarkers. Large-scale clinical studies are required to determine whether extracellular vesicle-associated miRNAs can reliably distinguish between different cardiovascular conditions, predict disease progression, or guide therapeutic decisions. Advances in high-throughput sequencing, proteomics, and bioinformatics will likely accelerate biomarker discovery in this field.

Moreover, continued research into extracellular vesicle-based therapeutic strategies holds considerable promise. Engineered extracellular vesicles may serve as effective delivery vehicles for miRNA mimics, anti-miRNA molecules, or other therapeutic agents targeting pathological pathways in cardiovascular diseases. However, challenges related to targeting specificity, large-scale production, safety, and regulatory considerations must be addressed before extracellular vesicle-based therapies can be widely implemented in clinical

settings.

Overall, a deeper understanding of extracellular vesicle-mediated intercellular communication is expected to provide important insights into the mechanisms underlying cardiovascular diseases. These advances may ultimately open new avenues for early diagnosis, disease monitoring, and the development of innovative therapeutic strategies.

9. Conclusion

Extracellular vesicles are key mediators of intercellular communication and play crucial roles in cardiovascular physiology and pathology. Through the transport of miRNAs and other bioactive molecules, extracellular vesicles regulate various biological processes involved in vascular homeostasis and disease development.

In particular, extracellular vesicle-associated miRNAs have emerged as important regulators in the pathogenesis of atherosclerosis and other cardiovascular diseases. Continued research in this field may lead to the development of novel diagnostic biomarkers and new therapeutic approaches.

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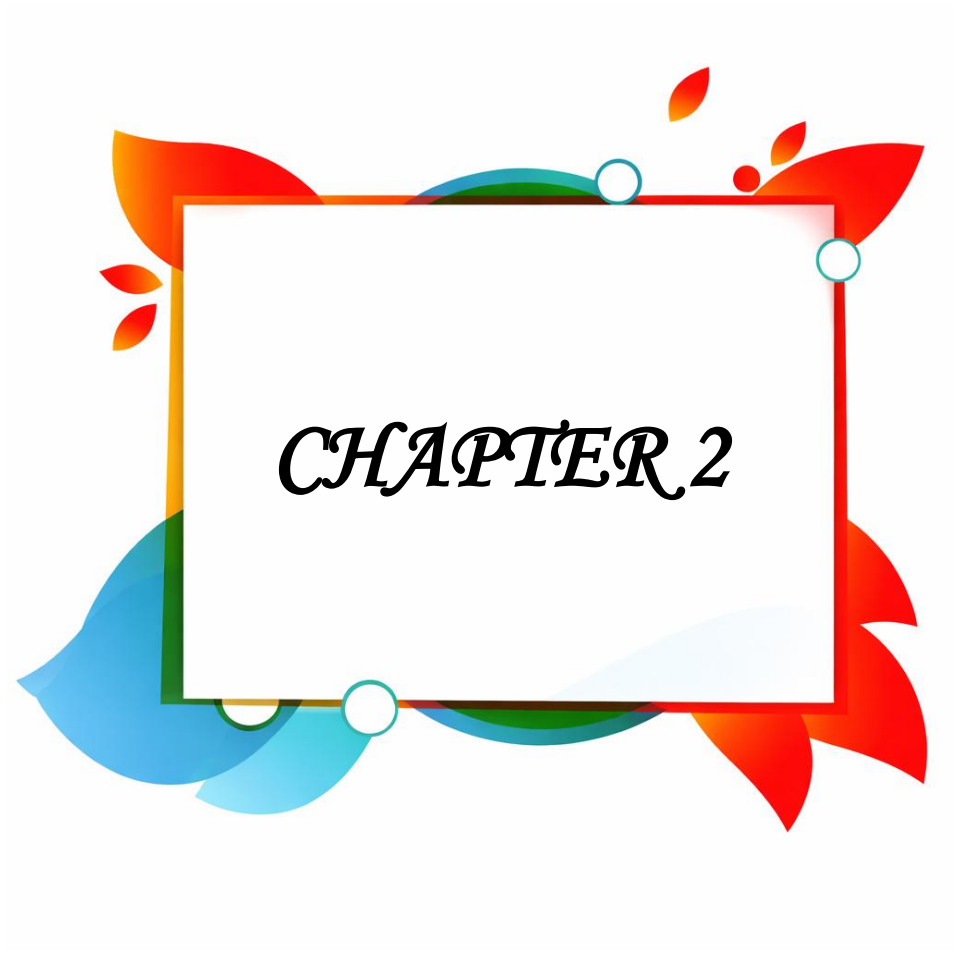
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New Horizons In Food And Health: Exopolysaccharides Of Lactic Acid Bacteria

Osman Eren¹

Exopolysaccharides

EPS are polysaccharide molecules composed of monosaccharide units linked via glycosidic bonds, which can adopt either linear or branched configurations. They are synthesized by a diverse range of organisms, including plants, fungi, cyanobacteria, vertebrates, invertebrates, and bacteria. These linear or branched polymers, formed through the homo or hetero condensation of monomers such as glucose, galactose, mannose, rhamnose, and fructose, can be further modified through the attachment of acidic or amino sugars, peptides and proteins, acyl groups, lipids, nucleic acids, or inorganic moieties such as sulfate and phosphate. EPS composed of a single type of sugar are classified as HoPS, whereas those containing multiple distinct sugar units are termed HePS. The primary function of EPS is to constitute the structural framework of the biofilm matrix, providing a protective barrier that shields cells from adverse environmental stresses, including heat, light, radiation, UV exposure, dehydration, and phagocytosis. Polysaccharides secreted by microorganisms can be categorized as intracellular storage polysaccharides (e.g., glycogen), capsular polysaccharides (CPS) covalently attached to the cell membrane or wall, or extracellular EPS, which interact weakly with or remain unbound to the cell surface (e.g., xanthan, alginate, cellulose). In bacteria, four principal EPS biosynthesis mechanisms have been identified: the Wzx/Wzy-dependent pathway, the ATP-binding cassette (ABC) transporter-dependent pathway, the synthase-dependent pathway, and extracellular synthesis. EPS have been reported to exhibit a wide spectrum of bioactivities, including anti-inflammatory, antioxidant, immunostimulatory, antitumor, prebiotic, and antithrombotic effects. Furthermore, EPS confer multiple functional properties in foods, acting as antimicrobial agents, emulsifiers, thickeners, and hydrocolloids. Owing to their biocompatibility, biodegradability, and low toxicity, EPS hold considerable potential for diverse applications across medical, food, and biotechnological industries, pharmacological processes, agriculture, cosmetics and hygiene, heavy metal adsorption, textile technologies, and thermal insulation. Consequently, research on EPS has increased substantially in recent years; however, their full range of

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effects and the precise mechanisms governing their secretion remain incompletely understood (Gürleyendağ 2006; Dertli et al., 2013; Ergene, 2015; Schmid et al., 2015; Kavitate et al., 2019; Devi et al., 2016; Tiwari et al., 2021 Eren & Şakiroğlu, 2025).

Lactic Acid Bacteria Exopolysaccharides (LAB-EPS)

Approximately 30 LAB species have been reported to synthesize EPS. LAB derived EPS are generally mucus like polysaccharides that are either loosely associated with the cell membrane or entirely unbound. The physicochemical properties and biosynthetic mechanisms of LAB EPS vary widely, but they are typically high-viscosity, water-soluble organic complexes exhibiting linear or branched structures, and can be classified as HoPS or HePS. HePS generally possess lower molecular weights and are synthesized intracellularly, whereas HoPS are characterized by higher molecular weights, extracellular synthesis, and higher production yields. HoPS are composed of neutral monosaccharides such as glucose (glucans), fructose (fructans), or galactose (polygalactans), and their functional properties are predominantly prebiotic. In contrast, HePS often contain charged groups and are more associated with immunomodulatory functions. Like EPS from other bacterial sources, LAB EPS can confer therapeutic benefits or enhance the technological and nutritional properties of food products (Figure 1-2). Moreover, owing to the GRAS status of LAB, their EPS are generally recognized as safe for consumption. (Torino et al., 2015; Lynch et al., 2018; Xu et al., 2019; Yang et al., 2023). HoPS are typically synthesized extracellularly through the catalytic activity of enzymes belonging to the glycosyl hydrolase family, with sucrose generally serving as the glycosyl donor. The specific enzyme involved depends on whether the polysaccharide is an α -glucan or a β -fructan, with glucansucrases or fructansucrases mediating the respective polymerization reactions. α -Glucans are produced by a limited number of LAB genera, including *Leuconostoc*, *Lactobacillus*, *Lactiplantibacillus*, *Limosilactobacillus*, *Lacticaseibacillus*, *Streptococcus*, *Pediococcus*, and *Weissella*. In contrast, β -glucans are synthesized intracellularly, catalyzed by glucosyltransferases that do not utilize sucrose as a substrate. β -Glucan production has been predominantly reported in bacteria isolated from alcoholic beverages, such as *Pediococcus* spp., *Lactiplantibacillus* spp., and *Oenococcus* spp. EPS secreted by *Leuconostoc* and *Weissella* species generally consist of linear α -D-glucan chains. Genes responsible for EPS biosynthesis in LAB may be located either on plasmids or chromosomal DNA, reflecting the diverse genetic regulation underlying polysaccharide production (Donot et al., 2012; Chapot-Chartier & Kulakauskas, 2014; Torino et al., 2015 Caggianiello et al., 2016; Prete et al., 2021; Liang et al., 2024). The biosynthesis of HePS is inherently more complex due to their intricate configurations. These polymers, which typically contain between 2 and 8 distinct monosaccharide units, can adopt linear or branched structures. The synthesis of repeating units occurs intracellularly within the cytoplasm, whereas

polymerization reactions take place extracellularly. In contrast, HoPS are predominantly produced by LAB species such as *Lactocaseibacillus casei*, *Latilactobacillus sakei*, *Lactocaseibacillus rhamnosus*, *Lactobacillus acidophilus*, and *Lactobacillus delbrueckii* subsp. *bulgaricus* (S. Liang et al., 2024).

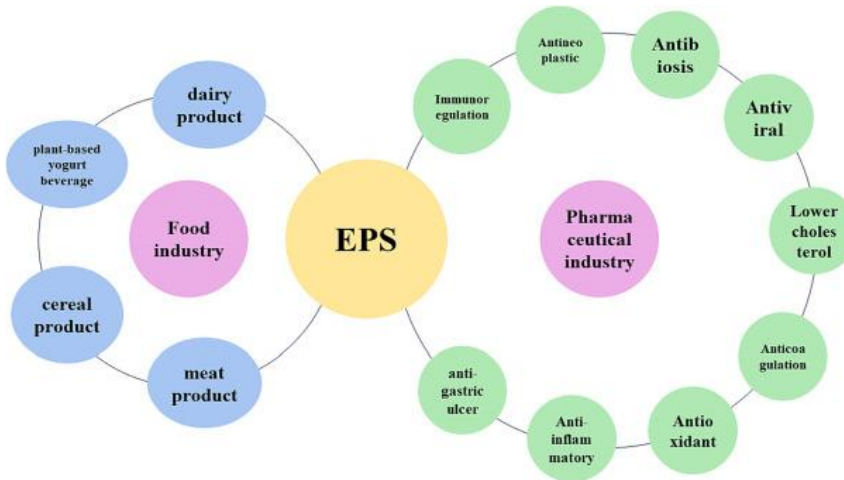


Figure 1. Applications of LAB Exopolysaccharides in Various Fields (Zhang et al., 2024)

Application of LAB Exopolysaccharides in Food Biotechnology

Currently, the food industry predominantly utilizes plant- and animal derived EPS due to the suboptimal yields associated with bacterial EPS production, which consequently increases production costs. Nevertheless, the impact of bacterial EPS, particularly when produced in situ, is often greater, and their use can reduce the need for additional additives, thereby enhancing their appeal. In summary, owing to their technological and physiological functionalities, LAB derived EPS are increasingly employed as natural and safe food additives that improve the technological properties of food products (Caggianiello et al., 2016; Zhang, Liu, Liang, et al., 2024). LAB-derived EPS can be employed in the food industry for a variety of purposes, including as gelling agents, emulsifiers, stabilizers, hydrocolloids, and food preservatives with antibacterial and antifungal properties. By modulating rheological properties, firmness, and viscosity, these polymers can alter and enhance the texture, sensory attributes, flavor, and overall palatability of food products, while simultaneously extending their shelf life (Dikmen et al., 2023; Hakim et al., 2023; Liang et al., 2024; Zhang, Liu et al., 2024).

Application of LAB-Derived EPS in Dairy Products

In particular, in situ EPS production in dairy products enhances the functional and sensory properties of fermented milk. One study reported that EPS synthesized in situ improved the water-holding capacity, texture, and overall

sensory attributes of yogurt (Sasikumar et al., 2017). Whey separation in yogurt occurs due to weakening of the gel network, resulting in an undesirable appearance. Although whey is often discarded because of its unpalatable taste, it contains valuable vitamins. EPS can enhance both the quality indicators and nutritional value of yogurt, and its hydrocolloid properties help reduce whey separation. This promotes increased protein–protein interactions, leading to the formation of a denser and more cohesive gel network (Cao et al., 2021; Ge, Bao, Feng, et al., 2022; Ge, Bao, Li, et al., 2022; Ng et al., 2022; Tiwari et al., 2021; Zhang, Liu, Tang, et al., 2024; Zhang, Tang, et al., 2024; X. Zhao & Liang, 2022). In another study, the effects of EPS produced by *Lactiplantibacillus plantarum* HMX2 on yogurt quality indicators were evaluated, either in combination with whey protein (PWP) or alone. The results demonstrated that the EPS–PWP mixture yielded the most favorable outcomes in terms of viscosity, adhesiveness, cohesiveness, water-holding capacity, and viable cell counts, highlighting its positive impact on both the quality and nutritional attributes of yogurt (G. Hu et al., 2025). When used in a 1:1 ratio with a commercial yogurt starter, the EPS-producing *Lactiplantibacillus plantarum* DPA1C enhanced yogurt viscosity and increased in situ EPS production. Furthermore, this strain promoted the growth of the probiotic *Bifidobacterium* by suppressing pathogenic bacteria in the gut (Wang et al., 2025). Due to limitations in the water-binding and hydrophilic properties of casein and lactoglobulin, dairy products may exhibit whey separation, leading to rheological defects that can reduce both shelf life and consumer acceptance. While the addition of stabilizing additives can mitigate these issues, the use of starter cultures capable of in situ EPS production offers a promising strategy to address this problem naturally (Zhang et al., 2024).

Researchers isolated a high EPS-producing LAB strain from kefir grains and employed it as a starter culture for yogurt production. The study evaluated yogurt EPS content, viable cell counts, titratable acidity, pH, water-holding capacity (WHC), texture, rheological properties, microstructure, volatile flavor compounds, and sensory attributes. Overall, the results indicated that the strain could be effectively utilized as a starter, producing significant improvements across all measured parameters (Wang et al., 2023). In a separate study, the EPS-producing strains *Limosilactobacillus fermentum* Lf2 (Lf2) and *Bifidobacterium animalis* INL1 (INL1) were employed as starter cultures for yogurt production. The findings indicated that both strains contributed significantly to improvements in yogurt rheology, water-holding capacity, and bioactive peptide content, as well as other quality parameters such as organic acid production, carbohydrate levels, and aminopeptidase activity (Ale et al., 2023). Researchers utilized cheese whey, a dairy by-product, as a culture medium for EPS production via *Chlorella vulgaris*. The study reported efficient EPS synthesis, with the resulting polymers exhibiting pronounced pseudoplastic properties. Additionally, the researchers noted that other quality attributes of the EPS were comparable to those of

commercial products, highlighting its potential applicability in the food industry (Pessôa et al., 2024). In skim milk-based bioprocesses, researchers characterized EPS produced by *Lactobacillus rhamnosus* and reported that this polymer could enhance texture in milk-based products (Copeland et al., 2025). Through optimization processes, researchers reported an increased in situ yield of EPS produced by *Limosilactobacillus fermentum* CFW6, isolated from pineapple pulp. The findings indicated that the EPS exhibits prebiotic functionality, demonstrates pseudoplastic behavior in dairy products, and significantly enhances quality parameters such as viscosity, consistency, and acidity in plant-based milk products (Mete et al., 2025). Although the observed yield suggests limited immediate commercial potential, the results indicate that with improved optimization and conditions, the EPS could be commercially viable. LAB are also employed in other fermented dairy products, such as cheese. Studies have demonstrated that LAB-derived EPS can increase moisture content in cheese, reduce the total quantity of fatty acids without altering their composition, and, in a separate investigation, EPS extracted from cheese exhibited high antioxidant capacity and DNA repair activity (Oluk & Güven, 2015; Şanlı et al., 2018; İnanan et al., 2024). A high moisture content in cheese is generally undesirable, as it can negatively affect texture, appearance, and flavor in this protein-rich product, and may also compromise microbial stability. Therefore, EPS do not always exert beneficial effects. Research has reported that EPS had no significant impact on butter (Akgül, 2020), conferred positive effects in ice cream (Dertli et al., 2016; Yasar & Yeydem, 2023), and exhibited variable influences on ayran depending on the EPS type and bacterial strain used (Güzeler et al., 2006; Yilmaz et al., 2015; Ergin, 2021). Researchers investigating EPS produced in milk by the LAB *Lactobacillus rhamnosus* ZFM231 reported that all four isolated EPS variants possess potential applications in both the food and pharmaceutical industries (S. M. Hu et al., 2021).

The effects of EPS have also been investigated in other fermented dairy products, including cheese, butter, ice cream, and ayran. However, a review of the scientific literature indicates that, apart from yogurt, EPS effects in these products have been relatively underexplored. Comprehensive evaluation of both the positive and negative impacts of EPS in these matrices requires further research.

Application of LAB-Derived EPS in Cereal Products

Although the application and effects of EPS have predominantly focused on dairy biotechnology, their functional properties also present considerable potential for utilization in other sectors of the food industry and biotechnology. The hydrocolloid nature of EPS makes it a promising alternative to chemical hydrocolloids in bakery products. Studies have generally reported that EPS can increase the specific volume of bread, improve texture, reduce crumb hardness,

and slow staling. Additionally, EPS can lower pH, enhance the rheological and viscoelastic properties of sourdough, reduce baking losses, and contribute to a desirable crumb structure. The addition of sucrose to the dough can further promote the production of EPS, such as glucans, thereby improving dough quality (Hakim et al., 2023; Lynch, Coffey, et al., 2018; Milanovic et al., 2020; D. Xu et al., 2020; J. Zhang et al., 2023).

Application of LAB-Derived EPS in Other Food Sectors

EPSs can improve dough formation and quality not only in wheat flour but also in legume flours. The lack of gluten in legume flours can technologically hinder the preparation of dough with the desired consistency and subsequent bread production. The hydrocolloid properties of EPSs can facilitate overcoming these challenges. Studies have reported that EPSs enable the production of processable dough from legumes such as lentils and chickpeas, as well as from plant seed flours like amaranth and quinoa (Galli et al., 2020; Perri et al., 2021; Valerio et al., 2020; Y. Xu, Coda, et al., 2019). Researchers employing EPS-producing strains have recommended the use of EPS in bread production due to its benefits for baking technology, cost efficiency, and shelf life. However, the study also indicated that EPS may reduce the formation of resistant starch (RS). While this effect is favorable in terms of extending shelf life, RS is a highly beneficial compound for gut health (Buksa et al., 2021; Kübra et al., 2025).

Researchers using dextran produced in situ by *Leuconostoc citreum* DSM 5577 as a starter culture in broken rice protein yogurt reported positive outcomes and concluded that this strain is suitable for application in such products (Lu et al., 2023).

EPS produced by *Lactobacillus pentosus* YY-112, isolated from wax apple, has been demonstrated to confer beneficial effects on the gut microbiota, indicating the strain's potential as a probiotic. This bacterium exhibits moderate sensitivity to gastric acidity and bile salts, and contributes to gastrointestinal health by modulating the acetic acid to probiotic acid ratio (Wang et al., 2021). Researchers utilizing psychrophilic, EPS-producing *Lactiplantibacillus plantarum* reported that the strain significantly improved the quality indicators and antioxidant activity of oat silage compared to commercial starter cultures (Su et al., 2024). A study reported that EPS derived from *Lactobacillus plantarum* reduced the uptake and toxicity of cadmium, a harmful heavy metal, in rice seedlings (Li et al., 2022).

Researchers isolated EPS from *Lactiplantibacillus plantarum* HDC-01 and characterized its properties. The study reported that the EPS exhibited good thermal stability, water-holding capacity, and milk coagulation ability, while also supporting the growth of probiotics, indicating its potential application as a prebiotic in both food and medical contexts (Yu et al., 2023).

In a study, EPS was extracted from *Lactiplantibacillus plantarum* HMX2, isolated from sauerkraut, and its effects were evaluated in diets for turbot (*Scophthalmus maximus*). The results demonstrated that dietary supplementation with HMX2-derived EPS promoted growth, enhanced antioxidant activity, improved digestive and immune capacities, and actively modulated the gut microbiota of juvenile turbot. (G. Hu et al., 2023)

Therapeutic Effects of LAB Exopolysaccharides EPS

Functional Roles of Exopolysaccharides in Host Health

EPS can exhibit highly variable composition and configuration depending on the culture medium in which the bacteria are inoculated, the environmental conditions, and the source from which they are derived. Consequently, they may exert diverse therapeutic effects. EPS play significant roles in glycemic modulation, the absorption of minerals such as calcium and magnesium, antioxidant and anticarcinogenic activities, enhancement of overall host health, modulation of the GI system, cytoprotective effects on intestinal epithelial tissue, and cholesterol homeostasis. Additionally, EPS can act as prebiotics, immunomodulators, antibiofilm agents, antimicrobials, and facilitate drug delivery, thereby contributing to health promotion. Studies have reported that EPS produced by LAB may have potential applications in anti-inflammatory therapies and the treatment of ibuprofen-induced gastric ulcers. Furthermore, EPS are well recognized for their antioxidant properties, and sulfation of EPS has been reported to enhance their antioxidant activity (Aliouche et al., 2024; Caggianiello et al., 2016; Jurášková et al., 2025; Kavitake et al., 2022; Korcz et al., 2018; Kusmiati et al., 2025; Z. Liu et al., 2019; Prete et al., 2024; Rahbar Saadat et al., 2019; Y. Wang et al., 2023) .

Modulation of Gut Health and Metabolism by EPS-Producing LAB

Researchers utilizing milk fermented with EPS-producing *Weissella* reported that EPS-enriched fermented milk may be effective in alleviating chronic constipation. Additionally, marginally significant effects were observed in pathogen inhibition within the gut and in the proliferation of probiotic strains (Jin et al., 2023). EPS produced by *Leuconostoc mesenteroides* has been shown to modulate glucose metabolism and energy homeostasis, while exerting beneficial effects on the composition of the gut microbiota (Miyamoto et al., 2023).

Binding and Mitigation of Aflatoxins by EPS-Producing LAB

Aflatoxins are toxigenic organic molecules produced by fungi, exhibiting immunosuppressive and carcinogenic effects. Their presence in food products poses significant health risks. Due to the inherent stability of aflatoxin M1, conventional milk-processing methods are largely ineffective in eliminating this toxin. Therefore, novel approaches are required to mitigate its presence for the protection of human and animal health. Researchers have reported that EPS

isolated from kefir can bind aflatoxin M1, indicating its potential application in the filtration and removal of this hazardous compound (Hernández-Martínez et al., 2025).

Histamine Mitigation by EPS-Producing LAB

Histamine poisoning primarily arises from the decarboxylation of histidine catalyzed by histidine decarboxylase enzymes produced by microorganisms such as *Morganella morganii*, *Klebsiella oxytoca*, *Photobacterium phosphoreum*, and *Photobacterium damsela*. Although these bacteria are inhibited at cooking temperatures, histamine itself is thermotolerant and remains undegraded. Consequently, its presence constitutes a significant health risk, particularly for immunocompromised individuals (Visciano et al., 2014; Bjornsdottir-Butler et al., 2016). In a study, *Lactobacillus paracasei* TOKAI 65 was reported to produce exopolysaccharides capable of binding histamine, indicating its potential application as a strain for mitigating histamine poisoning (Kinoshita et al., 2021).

Inhibition of Pathogenic Bacteria and Biofilms by EPS-Producing LAB

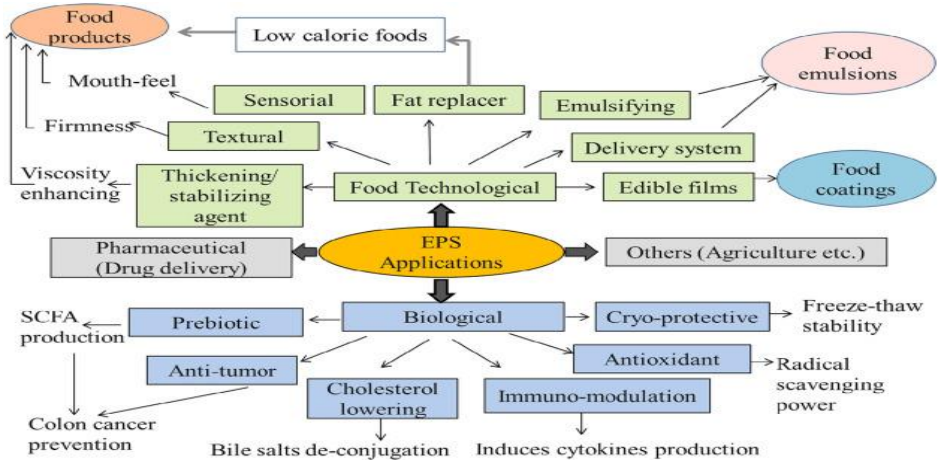
Researchers isolating EPS-producing LAB strains from spontaneously fermented foods and beverages reported that these strains were resistant to simulated gastrointestinal conditions, and that their exopolysaccharides possessed the ability to inhibit and disrupt pathogenic biofilms (Abid et al., 2018). Researchers fermented soy flour using EPS-producing LAB to investigate the effect of EPS-enriched dough on the adhesion capacity of Enterotoxigenic *Escherichia coli* (ETEC). The results indicated that the presence of EPS mitigated the pathogenic effects of ETEC. ETEC is an adherent pathogen capable of colonizing the epithelium and causing gastrointestinal infections and diarrhea (Pramudito et al., 2025).

Antiviral Potential of LAB-Derived EPS Against Adenoviruses

Despite the implementation of vaccination and chemoprevention strategies, adenoviruses continue to be highly prevalent. These viral agents can cause infectious disorders affecting the keratoconjunctiva, respiratory tract, urogenital system, and GI tract. Although such infections are often self-limiting, they may lead to more severe health complications in infants, pregnant individuals, the elderly, and immunocompromised populations (Ghebremedhin, 2014; Robinson et al., 2013). Researchers investigating the antiviral activity of EPS produced by lactic acid bacteria belonging to the genera *Pediococcus*, *Leuconostoc*, and *Lactobacillus* against human adenovirus type 5 have reported that these EPS could potentially serve as anti-adenoviral agents (Biliavska et al., 2019).

Therapeutic Potential of EPS in Heavy Metal Detoxification

A rapidly emerging area of interest regarding the therapeutic effects of EPS is their capacity for heavy metal binding and chelation. Heavy metal contamination is an increasingly pressing global concern. Accumulation of heavy metals in living organisms can lead to dysfunction of the intestinal epithelial barrier, acute and chronic poisoning, and toxicity in various organs and tissues. One alternative strategy for heavy metal detoxification involves the use of EPS produced by



LAB. EPS, which play a role in heavy metal adsorption in aqueous environments, can also serve this function in vivo. Studies have demonstrated that EPS exhibit significant efficacy in the adsorption of heavy metals such as cadmium and lead (Zhai et al., 2016; Yi et al., 2017; Brdarić et al., 2021, 2023; Wang et al., 2023).

Figure 2. Functions of EPS (Tiwari et al., 2021).

Conclusion and Future Perspectives

In recent years, rising consumer living standards have markedly increased demand for “clean-label” foods, functional products, and therapeutic foods. Although their exact nature was not fully understood, lactic acid bacteria (LAB) have been used by humans for millennia, primarily in fermented food production and various other applications. LAB constitute a critical industrial and probiotic bacterial group in biotechnological processes, primarily aimed at converting edible raw materials into value-added products. Through their homofermentative or heterofermentative capabilities, they can efficiently ferment a wide array of substrates, rendering them applicable across numerous sectors, particularly in the food industry. LAB serve versatile roles as fermentation starters, quality-enhancing fermentation agents, probiotics, postbiotics, therapeutic or pharmaceutical adjuncts, nutritional supplements, and even in cosmetic applications. Their ubiquitous presence across ecological niches facilitates relatively easy isolation, and a significant portion of these bacteria thrive under

anaerobic incubation conditions. LAB growth can be supported using general media such as MRS and M17, as well as selective or modified media tailored for specific purposes. Typically mesophilic, their optimal growth occurs within slightly acidic to near-neutral pH ranges. Their acidophilic nature and the acidic character of some metabolites contribute to the inhibition of pathogens in the gastrointestinal tract of humans and animals, while the production of diverse metabolites with different functionalities opens broad applications in food biotechnology, medicine, cosmetics, and pharmacology.

Among the various metabolites with therapeutic and nutritional value, exopolysaccharides (EPS) represent one of the most significant. Widely utilized across medicine and food biotechnology, EPS consist primarily of linear or variably branched chains of monosaccharides. The inclusion of additional molecular components within their complex structures confers multifunctionality. EPS synthesis is a biopolymer production process that varies according to the inoculated strain, environmental conditions, and culture parameters. HoPS-type EPS predominantly function as prebiotic agents, whereas HePS exhibit more pronounced therapeutic effects. In this review, the contributions of EPS in food and medical applications have been discussed; however, the complete range of effects, mechanistic pathways, synthesis processes, and detailed functionalities of these complex molecules remain incompletely elucidated.

A major barrier to industrial application is production yield. Despite EPS being obtainable from numerous bacterial strains, industrial feasibility generally requires yields exceeding 10-15 g/L. Optimized fermentation conditions, directed and random evolution approaches, co culturing strategies that facilitate synergistic interactions, and genetic and metabolic engineering can enhance yields, thereby enabling large-scale industrial production. Moreover, although EPS derived from living cells offer advantages over synthetic alternatives, comprehensive cytotoxicity and genotoxicity analyses are necessary. EPS obtained from pathogenic microorganisms may pose toxicological risks, underscoring the importance of using GRAS- or QPS-status strains. While beneficial effects of EPS have been demonstrated in simulated models, animal experiments, or in silico studies, their translation to human physiology may differ. Particularly, the manifestation of therapeutic effects and biochemical properties of EPS, once isolated and characterized in silico, within live organisms and complex food matrices remains uncertain. Interactions between these reactive EPS molecules and endogenous complexes, enzymes, and other cytosolic components generated through anabolic and catabolic pathways further complicate the prediction of physiological outcomes. Therefore, detailed and systematic studies are essential to fully understand the effects of EPS on human physiology.

Although the in situ production of EPS generally exhibits more pronounced effects in food matrices compared to in silico predictions, their highly variable nature and sensitivity to environmental conditions complicate standardization. While EPS frequently exert positive effects, undesired outcomes can occasionally arise, warranting further investigation. Literature indicates that EPS effects and particularly in situ production effects have been predominantly studied in dairy products, especially yogurt. Consequently, comprehensive studies are needed to elucidate their impacts on other fermented dairy products and across different food groups, including cereals. While the final effect of in situ EPS production is often more observable, the underlying mechanisms are less well characterized compared to in silico studies, emphasizing the need for detailed and systematic research in this area.

The diverse physicochemical properties of EPS preclude limiting their relevance to therapeutic, nutraceutical, or food applications. Modern tools, such as artificial intelligence (AI) systems, allow for more precise molecular design and predictive functional assessments without laboratory experimentation. By leveraging AI, bioinspired novel biomolecules can be designed, and their mechanisms of action can be elucidated rapidly and efficiently. Such approaches enhance both yield and time efficiency and contribute to the principles of a circular economy. This opens possibilities for EPS to be employed more functionally and efficiently in applications such as thermal insulation materials, bioplastics, or cleaning industry products. Hence, these complex organic molecules require a multidisciplinary investigation to explore their potential applications across industrial, technological, and chemical domains. Contributions from computer science, physics, textiles, surface chemistry, cleaning chemistry, and social sciences, including economics, are vital for advancing this field.

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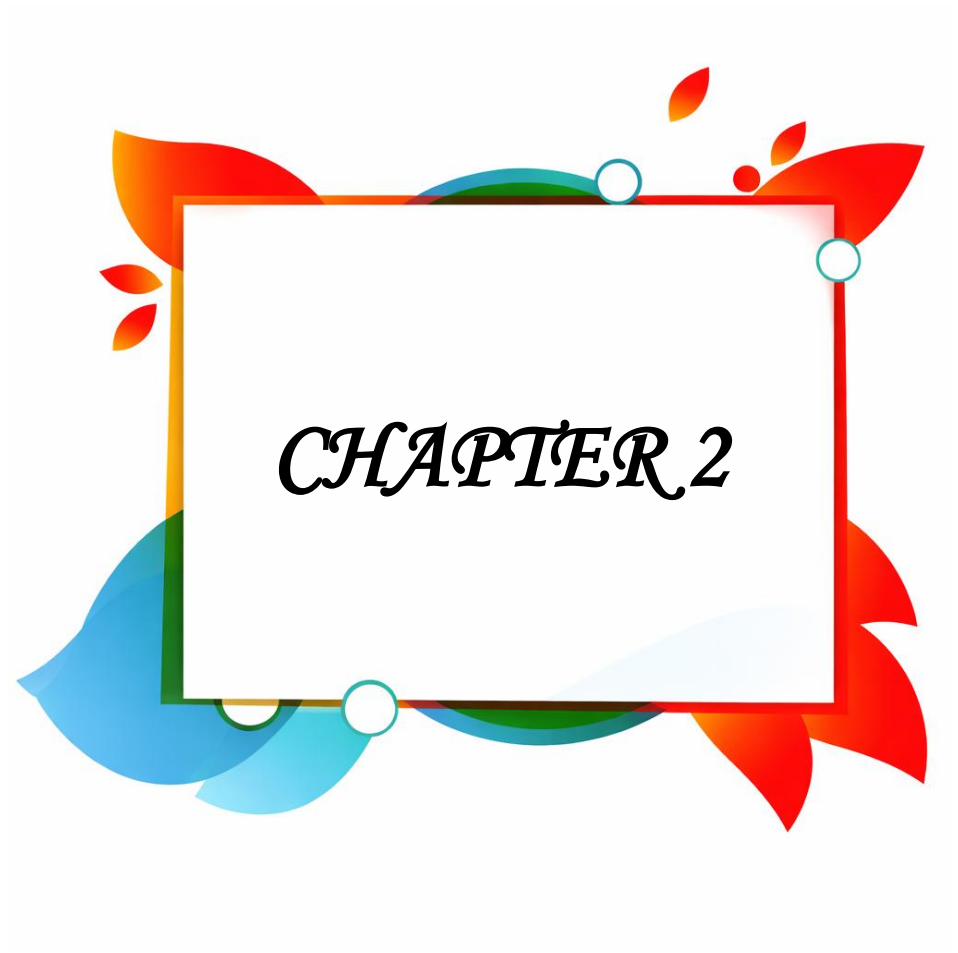
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Usage of Advanced Machine Learning Tools for Treatment Optimization Within the Framework of Personalized Medication Management

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Introduction

Machine learning commonly known as ‘ML’ is fast becoming a tool for optimizing treatment and patients’ specific needs for treating their illnesses. Most image diagnostic tests can be run by the (Kosorok & Moodie, 2016; Zhao, 2022) ML algorithms to increase the accuracy of diagnosing the diseases, providing individualized treatment tips, and in general, increasing the effects of treatment for most patients (Deepak Kumar et al., 2024). In pharmacometrics, the use of ML is advocated for data pre-processing, development of hypotheses, building predictive models, and model checking ((Janssen et al., 2022). EMRs are more and more leveraged with ML to help drug development and precision medicine investigations (Shinozaki, 2020). Dynamic treatment regimens (DTRs) and adaptive treatment strategies (ATSS) are important conceptual tools for delivering personalized medicine. Real-world, individual treatment effects are the goal for which ML methods are currently being developed, although there are some limitations (Bica et al., 2021). The use of ML in TM is enhancing target identification, patient stratification and dosing (Terranova et al., 2021). Both findings suggest that ML methods have significant potential in the context of immune-mediated chronic inflammatory diseases across future prospects of prediction, identification, programming, and treatment plans (Peng et al., 2021).

Machine learning has emerged as a transformative technological advancement that is significantly reshaping personalized medicine and treatment approaches. Approximately in the year 2024, advanced functions of ML algorithms will be able to accurately diagnose a patient based on large amounts of data collected regarding the patient. Smart hospitals where patients have IoMT devices would

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benefit from individual medication management to enhance safety by utilizing ML (Rawas, 2024). In particular, deep learning and ensemble methods are valuable for anticipating the treatment response of a specific patient to various therapies. Such approaches have been used effectively in the experimental combined stimulation and medication management for Parkinson's disease (Gutowski et al., 2023; Shamir et al., 2015) and personalized drug intake schedule development (Gutowski et al., 2023). It is also important to note that the concept of heterogeneous treatment effects (HTEs) can be effectively explored using machine learning approaches in clinical trials (Curth et al., 2024; Inoue et al., 2024), as well as in therapeutic drug monitoring and model-informed precision dosing.

Personalized Medicine (PM) is a newly developed model of providing medical treatment based on the patient's specifications. This form of medicine is in contrast to traditional medicine where every patient with a given disease is given the same treatment regime. This has developed in the recent past especially with development in technology like genomics, pharmacogenomics and even machine learning (Collins & Varmus, 2015). The increasing cases of personalized treatment are caused by the fact that people experience varying reactions in terms of diseases, genes differences and organization of human body. For example, genetic factors play a role in the patient's capacity to metabolize the drug or otherwise, and therefore the drug may work in one patient while recording different side effects in another patient. This technique seeks to ensure that the drug most suited to a particular patient and the right dose, is administered to him or her to enhance positive results and reduce the side effects (Ginsburg & Phillips, 2018). On this process, pharmacogenomics which focuses on how a person's genotype will influence their phenotype when treated with medicine is important since health care can use genetic tests to expect a patient's response to drugs before beginning the treatment process.

The Challenges of Traditional Treatment Approaches in the sector stem mainly from inter-individual variation regarding usual treatments and constraints inherent to a generalized treatment model. One limitation that flows from embracing traditional medicine is that it uses an "across-the-board" approach when treating its patients, which means that it expects all patients who are suffering from particular disease or symptoms will react in the same manner to a particular treatment. However, this assumption disregards crucial variations in genetic makeup, physiological response, personal habits and contours of the environment in which people live that can modulate both the effectiveness and the safety of a particular approach or intervention (Ginsburg & Phillips, 2018). One of the main problems of applying conventional methods for interacting with the patient is variability in the patient response. For instance, drugs which are helpful to some patients are less effective or toxic to others. Genetic features are

the main cause of this variation; variations in genes that code for drug-metabolizing enzymes, drug receptors, and drug carriers cause variation in the pharmacokinetics and pharmacodynamics of the drugs outcome (Gottesman, 2026). For instance, genetic polymorphisms that affect CYP2C9 and VKORC1 genes affect both the metabolism of the warfarin, an anticoagulant drug, and the correct dose to administer to prevent excessive bleeding or formation of blood clots (Johnson, Caudle, et al., 2017) .

The Use of Machine Learning (ML) in enhancing healthcare is critical in medical treatments by integrating data analytic tools to fashion special treatments. Unlike the traditional clinical algorithm, the ML models are capable of processing and analyzing large and complicated data to understand special cases of a certain patient and then produce specific solutions for this patient (Topol, 2019). Data mining, which consists of supervised mining, unsupervised mining, and reinforcement learning, can forecast the course of the illness, identify the right drug to use in treating a patient, and identify patients most likely to react badly to certain medications. There are two broad classifications in supervised learning models in ML of predicting future occurrences using known patterns from earlier judicious data: one enables the anticipation of the reaction from individual subjects, which is helpful in the medical field where healthcare specialists can gauge the reaction of patients to some treatments. For instance, determining the appropriate chemotherapy regimen for a cancer patient will involve data about the patient's past record on genetic makeup, tumor type and prior treatment history. Such predictive models assist doctors in choosing the best treatments avoiding trial and error approaches and enhancing outcomes among patients (Shah et al., 2019). Likewise, other types of learning algorithms like decision tree and support vector machines (SVM) perform categorization of patients according to the probability of the patients responding to certain treatments, so that more precise treatment procedures are assigned to the patients.

Furthermore, unsupervised learning methods can identify potential structures in patient data that are essential for subdividing the patient populations and discovering new subtypes of diseases which need different treatments. For example, machine learning based algorithms like K-means can cluster patients according to clinical characteristics and the resulting clusters might unveil latent subtypes of the disease and help designing individualized treatment strategy (Esteva et al., 2022). Also, the use of reinforcement learning for creating decision-making adaptations for the treatment procedure is being discussed, which means that the model learns the right sequence of treating the patient by updating the list of treatments depending on the patient's response through the data storage system. Such approaches have been revealed to increase effectiveness of the decisions made, thus transforming the delivery of personalized care (Yu et al., 2022).

It is therefore making dentists change from general treatment procedures resulting from standard treatment protocols to personal treatment plans derived from each patient's information in relation to his/her treatment hence improving on patient outcomes, adherence to the treatment plan and satisfaction.

2. Literature Review

Personalized medicine as a field has been steadily growing due to the development of new technologies including ML and AI. Most traditional treatments practices do not consider the genetic and physiological variations of subject patients, and this aspect can partly explain why treatment results cannot be uniform. Table1 represents a summary table article from the literature that focused on personalized medications. In (Bica et al., 2021) and (Yi et al., 2024) both linked ML as a means of solving such issues from observational data and maneuvering actual clinical data for individual treatment effect (ITE). These involved Bayesian Additive Regression Trees (BART), Causal Forests as well as Extreme Gradient Boosting (XGBoost) to identify other accurate prediction models for individual level interventions. Such an approach is effective in sorting patient-specific issues in relation to the best treatment methods in the provision of health care services.

Another important area where the results of ML application are felt in personalized medicine is in the management of chronic and autoimmune diseases. For instance, (Peng et al., 2021) as well as (Ejike Innocent Nwankwo et al., 2024) created an ML application for rheumatoid arthritis and multiple sclerosis. These studies used such techniques as logistic regression, the random forest, and recurrent neural networks (RNNs) to forecast the patients' responses and distinguish the disease subtypes for corresponding personalized therapy. The use of various sources of patient data such as genomics and proteomics data has proved to enhance therapy plans as well as target treatment to one's ability. That said, the problem regarding data quality, ethical concerns, and model validation still remains, which either hampers or delays the application of these approaches.

(Sarkar et al., 2023) and (Thakur et al., 2024) dedicated their work towards utilizing ML for improving the drug discovery and development. These studies showed that the use of a number of ML classification techniques such as Random Forests, SVM, and Deep Neural Networks could be incorporated to fast-track the prediction of new drug targets and the enhancement of drug efficiency. These ML techniques ease the process of drug development because it makes it possible for analysis of huge datasets like the bioactivity databases besides integrating biomedical data creating a pathway towards personalized medicine because drugs can be developed for special genetic makeup. It has proved helpful when it comes to the rationing of drugs and effects, they have on the body to ensure that the best care is offered. Biosensors and introduction of real-time data have been discussed by (Homescu, 2020; Thakur et al., 2024) to understand their role in developing

personal treatment. From these studies, the use of ML in biosensors and mobile sensing devices was explored to enhance medication management and enhance decision-making for patients' care. Depending on the patient data received in real time, these ML models can give individual recommendations that allow changing the treatment plan at the right time in response to the change in the condition of the patient. These together with the employment of biosensors, feature selection, and clustering algorithms are all major steps in the direction of a more flexible and individualized healthcare system.

Finally, the application of ML in clinical decision support systems and Treatment decisions were discussed by (Malviya et al., 2023; Plant & Barton, 2021; Verma et al., 2019). These studies made utilization of NLP techniques, ensemble techniques, and genomic data analysis in order to offer clinicians better accuracy when planning the treatment process. Through the use of such ML algorithms as the Support Vector Machines (SVM) and the deep learning algorithm the approaches seek to improve on patient screening, linking the patients to their right dosages and finally estimating their treatments outcomes. This not only reduces error-prone trial and error but also helps in better decision making in clinical practice for better health outcomes, which is the ultimate goal of this delivery modality in healthcare.

(Shinozaki, 2020; Zhao, 2022) examined how electronic medical records (EMRs) and dynamic treatment regimens (DTRs) can help enhance precision medicine and personalization treatment. (Shinozaki, 2020) observed that EMRs are increasingly being used together with ML models as an effective method to aid drug development and precision medicine projects, which is useful in utilizing patients' actual data. (Zhao, 2022) highlighted that applying DTRs and adaptive treatment strategies (ATs) for personalizing care, however, they pointed that treatment management should be dynamic and adjusted according to the patient's responses over the time. These studies point to the importance of understanding applicative data and continuous approaches to the assignation of the patients' care plans so that the interventional approaches to the patients' treatment are sensitive to their actual requirements. (Kosorok & Moodie, 2016) and (Terranova et al., 2021) dealt with theory and use of ML in translational medicine. (Kosorok & Moodie, 2016) offers a basic guide to adaptive treatment strategies and how they can help to use individual patient data and change via learning models to deliver unique medical care. Others elaborated this by showing how ML could improve target discovery, patient grouping and dosing in translational research according to (Terranova et al., 2021). These approaches have opened possibilities in providing customized treatment options in personalized medicine by employing sophisticated ML tools to discuss the patient subgroups and respective drug reactions.

The reviewed literature proves that the introduction of machine learning into a healthcare setting opens up vast improvements in the field of personalized medicine. From utilizing the EMRs to promoting the adaptive treatments, ML makes it easier to be more reactive in the care that is provided. The theoretical and practical applications described and discussed underscore how ML advances treatment individualization, drug discovery, and patient categorization and contributes to better patient outcomes. Raising quality of data, model verification, and ethical issues is critical to unleashing the potential of the mentioned approach in the healthcare system’s further evolution toward patient-centricity.

Table 1. Articles about Personalized Medications

	Article	Main Contribution	Methods	Data set
	(Bica et al., 2021)	Outlines the prospect and issues when applying observational data and Electronic Health Records (EHRs) in particular for building ML models for ITE. It emphasizes areas of ML for causal inference of RCTs and observational data, the prospects of merging pharmacometric plans with ML for personalized treatment suggestions.	Bayesian Additive Regression Trees (BART) Causal Forests Balancing Neural Networks Generative Adversarial Networks (GANITE) Counterfactual Recurrent Networks (CRN)	EHRs
	(Peng et al., 2021)	The article highlights the growing use of ML to improve how to understand and treat autoimmune diseases like rheumatoid arthritis and multiple sclerosis. By analyzing complex patient data, ML can help predict how well a patient will respond to a certain treatment, identify different subtypes of the disease, and even support drug development. The insights emphasize that while ML has the potential to revolutionize personalized healthcare, practical challenges such as data quality, ethics, and model validation still need to be addressed.	Supervised Learning: Algorithms like logistic regression and random forests Unsupervised Learning: Methods like clustering Deep Learning: Neural networks, such as RNNs and CNNs for image processing.	EHRs Omics Data Clinical Imaging
	(Sarkar et al., 2023)	The article explains how AI and ML are transforming drug discovery and development. By leveraging AI, pharmaceutical companies can analyze huge datasets more effectively, allowing them to discover promising drug candidates faster and with greater precision. The authors emphasize that integrating AI is a crucial step toward making the entire process	ML Algorithms: such as Random Forests, Support Vector Machines (SVM), and Deep Neural Networks are used to predict things like how drugs will interact with their targets and their potential side effects.	Molecular Data: Information about chemical and pharmacological properties Bioactivity Databases: Resources like

	Article	Main Contribution	Methods	Data set
		more efficient, from early drug discovery to clinical testing.	Deep Learning Techniques: like Convolutional Neural Networks (used to analyze images) and Recurrent Neural Networks (used for sequence prediction) are being applied to predict 3D protein structures and create entirely new drug candidates from scratch.	ChEMBL and PubChem, Clinical Data
	(Homescu, 2020)	Advanced ML tools enhance treatment optimization in personalized medication management by analyzing high-quality biosensor data, uncovering dependencies, and improving decision-making processes for tailored patient care.	NA	Mobile sensing and portable devices data Neuroimaging and Brain Machine Interface data
	(Deepak Kumar et al., 2024)	Advanced ML tools analyze diverse patient data to optimize treatment plans, enhancing personalized medication management by predicting illness likelihood and recommending tailored therapeutic options.	Feature Selection and Clustering Predictive Modeling: Combines multiple data sources to predict illnesses and recommend treatments.	Genetic information, medical history, lifestyle variables, EHRs Imaging data
	(Yi et al., 2024)	The study demonstrates that ML, particularly the Extreme Gradient Boost model, can effectively predict individual responses to antihypertensive treatments, enhancing personalized medication management and treatment optimization.	Least absolute shrinkage and selection operator method for variable selection Extreme Gradient Boost model for predicting individual BP responses	Pragmatic, cluster-randomized trial data on hypertension management in China visit records
	(Kaliappan et al., 2023)	The framework employs treatment optimization algorithms that utilize patient-specific data to enhance personalized medication management, improving the effectiveness of chronic pain interventions through advanced ML tools.	Predictive modeling and forecasting techniques. Treatment optimization algorithms for personalized strategies.	Historical pain records and lifestyle information. Genetic data.
	(Gaur et al., 2022)	Deep learning algorithms, such as CNNs and RNNs, optimize treatment in personalized medication management by analyzing extensive patient data to recommend tailored therapies based on individual characteristics.	Deep learning algorithms such as CNN, RNN, RBM, and autoencoders	Images Genomes

	Article	Main Contribution	Methods	Data set
			AI developments in the form of new and emerging technology	
	(Wang & Wang, 2023)	The AI model developed in this study utilizes advanced ML tools to enhance treatment optimization, enabling personalized medication management through accurate predictions and improved patient outcomes.	AI model trained on diverse historical medication data Advanced ML algorithms and techniques implemented	historical medication
	(Thakur et al., 2024)	Advanced ML tools enhance treatment optimization by integrating large-scale biomedical datasets, improving prediction accuracy of drug efficacy and patient responses in personalized medication management.	Advanced ML algorithms integrated with biomedical datasets Deep learning and reinforcement learning models used	Large-scale biomedical datasets for drug efficacy prediction. Real-world datasets
	(Verma et al., 2019)	Advanced ML tools optimize treatment by analyzing patient-specific data, identifying biomarkers, and predicting individual responses, thus enhancing personalized medication management strategies.	Deep learning techniques for predicting patient responses. Ensemble methods and reinforcement learning for treatment strategies.	Genomics and clinical history. omics data
	(Ejike Innocent Nwankwo et al., 2024)	Advanced ML tools analyze patient data to create tailored treatment plans, optimizing medication efficacy and minimizing adverse effects in personalized medication management.	AI analyzes medical data to tailor treatments for patients. ML identifies patterns and correlations in patient profiles.	Genetic information, EHRs, real-time health monitoring data
	(Bertsimas et al., 2020)	The study introduces ML4CAD, a personalized prescriptive algorithm utilizing advanced ML to optimize treatment for CAD patients, improving expected time to adverse events significantly.	Binary classifiers to detect adverse events due to CAD Regression models to estimate time from diagnosis to potential adverse event (TAE)	EHRs
	(Malviya et al., 2023)	Advanced ML tools, particularly support vector machines, enhance treatment optimization by analyzing patient responses to therapies, enabling personalized medication management through sentiment analysis and pharmaceutical data classification.	Support vector machines (SVM) for sentiment analysis. Random forest classification and linear SVC techniques.	Patient surveys Pharmaceutical data

	Article	Main Contribution	Methods	Data set
	(Plant & Barton, 2021)	ML approaches applied to genomic datasets can optimize treatment decisions in personalized medicine, moving away from trial and error to more precise patient-specific medication responses.	NA	Genomic dataset

3. Overview of Machine Learning Methods

3.1 Supervised Learning

In the supervised learning techniques, the learning process trains the model by using historical labeled data set to classify or predict new instances. The model will learn the relationship between the input and the output which is here the data label. Input represents the attributes or the features of certain instances, while the output represents the class or the target of each instance in the data set. To train the model properly, the model is sensitive to the quality of the used data set; missing values, outlier, and inaccurate data; data preprocessing steps should be solved before training the model. Data imbalance is another important factor in the supervised learning, the ideal case is to have same number of instances from each class (label); otherwise, the model will learn more from the class that has the majority of the instances which can lead to wrong classification that can affect the model’s performance.

In the health domain, supervised learning techniques play a major role in disease prediction such as diabetes, hypertension and cancer, classifying medical images (e.g., X-ray and CT scans), chronic disease predictions, patient segmentation, and finding personalized medicine by analyzing the demographic and electronic health records (EHRs) that contain visits, medications, and medical procedures.

Supervised learning is suitable for classification and regression assignments, in classification there are fixed number of classes (labels) and the model is asked to classify new unseen instances based on finding the relationship between input/output taken from the historical used data set. In regression, there are many types, the most used ones are linear regression, logistic regression, and

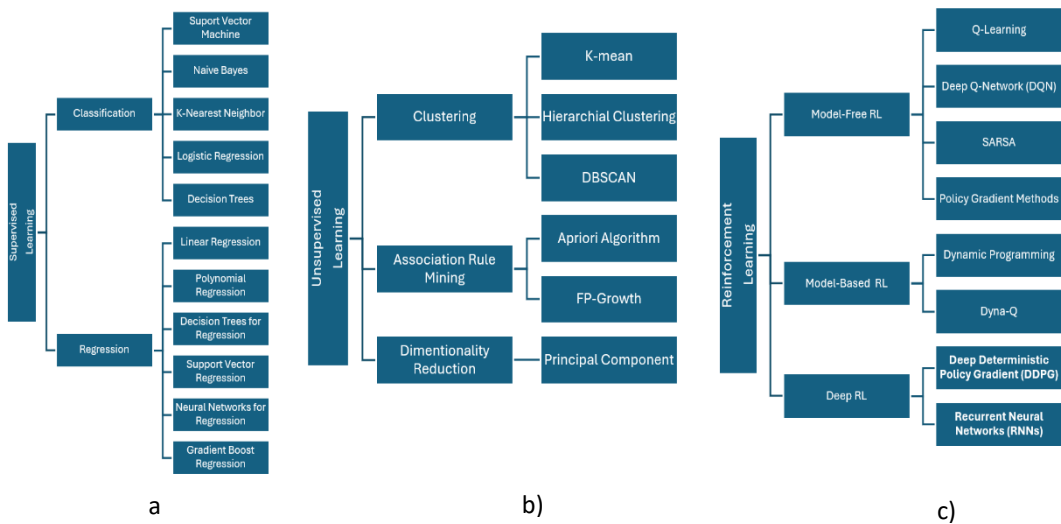


Figure 1. Machine Learning Methods

3.1.1 Classification Methods

Classification is a fundamental part of ML used to place data into defined categories or classes. Classification methods work as a backbone of many applications that involve decision making from input data – disease diagnosis, fraud identification, spam classification, and object recognition. Supervised classification techniques are used for classification of clear training data into particular classes and then used proposed classification knowledge to classify new unseen data into particular classes. It has methods such as decision tree, support vector machine, k-neighbor, logistic regression and naive bayes. All the methods can be used depending on the nature of the data and the type of problem which in this case is the classification problem. Classification techniques are still compulsory tools for both research and health care practitioners to predict outcomes and make right decisions, because of new opportunities of computations and data availability. Figure 1 includes the main classification methods that will be discussed in the next paragraphs.

Support Vector Machines (SVM): SVM is a classifier robust for its capability of choosing the right hyperplane of division of differentiated classes that leave the maximum margin between them. SVMs also deal with linear as well as non-linearly separable data by using kernel methods, which brings the data into higher dimensions. The algorithm picks the support vectors near the decision boundary that is required for optimum hyperplane. However, in the case of the SVM, tuning of the hyperparameters such as the kernel and penalty parameters (C) are necessary for its optimal effective. SVMs are applied in bioinformatics, image recognition and text categorization.

Naive Bayes Classifier: Naive Bayes classifier is a probabilistic model based on Bayes' theorem. Based on the class name, features are considered to be independent from each other, and thus, facilitates probability calculation. Naive Bayes classifiers do well when used in high dimensional feature space and they require little computation. It is common to use text classification like sentiment analysis and spam filtering. The Gaussian, Multinomial, and Bernoulli Naive Bayes classifier are suitable for all forms of data.

k-Nearest Neighbors (KNN): KNN is an instance-based learning approach that predicts cases that are nearest to well-classified instances from the feature space. KNN determines the distance between the new instance and the instances of its neighbors by means of techniques such as the Manhattan distance or the Euclidean distance. The class label will be determined among the new instance's k nearest neighbors based on majority vote. The optimal value of k should be chosen carefully since more values blur local tendencies while lesser values create more versatile and sensitive to noise models. KNN is usable for category and numerical data and has a simple concept and practice.

Logistic Regression (LR): LR is among the numerous popular Classification techniques that can be employed when we have nominal output variable, logistic regression models the correlation between input features and the probability of an instance belonging to a particular class. The logistic regression is traditionally applied in situations where the problem concerns the separation of data into two classes. In logistic regression a probability value is obtained from the linear regression output by passing it through a sigmoid function. The observed measures include accuracy, precision, recall, as well as F1 score to assess the given model. It trains itself through Maximum likelihood estimates.

Decision Trees (DTs): DTs are of the modeling algorithms in machine learning that fall under supervised learning technique that can be applied for classification and regression problems. In their workings, it is employed to iteratively divide the dataset into branches according to the features and decision outputs being described by the leaf's nodes. Decision trees are common because of the easy interpretability and because they can effectively pick up non-linearity in the data. There are keyways for splitting nodes depending on splitting criteria such as entropy (information gain) or gain impurity. One major issue in DTs is overfitting in which the model works well with training data but works badly and less efficiently with unseen test data that affects the generalization of the model. Pruning techniques are used to prevent overfitting.

3.1.2 Regression Methods

Regression is one of the most important concepts in ML and statistics where data is analyzed and put into practice for modeling a dependent variable with one or more independent variables. The main reasons for using regression are as follows: The main goal of regression is to predict the degree of some attribute

from the values of given features. Unlike classification, which is based on the discrete classes of output, regression methods are applied in the case where the output variable is quantitative. Some of the most used regression methods are linear, polynomial regression and support vector regression. Every method is therefore coined by special features that qualify it for a certain type of analysis based on the nature and size of data in question. Regression models are popular across many fields, including financial (i.e. stock price prediction), health (i.e. patient prognosis) and economic (i.e. market trend estimation). Unlike forecasts, these models are used for prediction and determination of dependence and interdependence between two factors, which is useful for decision-making and pattern identification. Figure 1 includes the main regression methods that will be discussed in the next paragraphs.

Linear Regression (LR): here we have two types the simple LR and the multiple LR. Simple LR estimates the association between two factors by mapping a straight line to the observations. It employs the least-square regression method in order to approximate the values in the model in order to try and fit the target variable in a linear model of the features a priori given, with the understanding that there is a linear relationship between the features and the target. Multiple LR is a slight development of simple regression straight-line model that uses more than one independent variable to predict only one dependent variable.

Polynomial Regression: As the term suggests polynomial regression expands into linear regression where non-linear relationships are investigated through the particular use of polynomial terms. It is used to determine the strength of the correlation of a dependent variable with respect to one or more independent variables as a straight line. It derives from linear regression and is used when the variables are related nonlinearly. What polynomial regression does is that, instead of finding the equation of a straight line, the model finds a curve that has a better fit to the data set.

Decision Trees for Regression: Regression tasks using continuous rather than categorical output variables can also be performed using decision trees. Regression trees divide the data depending on features to predict constant values. Minimizing mean squared error or mean absolute error is a common splitting criterion for regression trees. Pruning techniques, such as cost-complexity pruning, help prevent overfitting and simplify the tree structure. Multiple regression trees are used in ensemble approaches, such as random forests, to increase prediction accuracy.

Support Vector Regression (SVR): Support Vector regression is an extension of working principles of SVM for regression type problems. SVR seeks a function that fits the continuous output variable and input variables insofar as the tolerance for the error margin.

The linear model of SVR attempts to find the best line of fit in an attempt to determine the separating line while non-linear SVR maps the data to a higher dimensional feature space by using what is referred to as the kernel. There are many hyperparameters associated with SVR of which penalty parameter (C) and epsilon value need to be tuned for enhancing the performance. SVR applications can be found in diverse fields ranging from anomaly detection, energy load prediction to stock market prediction.

Neural Networks for Regression: Neural networks are powerful models capable of identifying multivariate relationship between input and output. In regression problems, all that the neural networks seek to do is to learn a mapping from the input space into the output space of real valued numbers. Interconnected artificial neurons, commonly referred as feed-forward neural networks, have several uses. Non-linear transformations such as Sigmoid, ReLU or tanh etc. are used to include the non-linearities into the models. Based on the established discrepancy between the output values as predicted by the trained ANN and the actual output values, adjusted of the network weights is taken using the so-called backpropagation technique. The problem of overfitting can be managed by adopting certain regularization measures including – dropout method and L1/L2 norm.

Gradient Boosting Regression: The gradient boosting regression is an ensemble learning technique which by combining several weak regression models fused generally decision tree creates a better predictability model. This method works by performing multiple base models on further on succeeding residual of preceding base models. Other boosting parameters such as the learning rate and the number of trees has to be set to the optimum level for it to perform to the maximum. Other complex incline enhancing tactics including the XGBoost and LightGBM partially enhance the regression accuracy together with computational efficiency.

3.2 Unsupervised Learning

In unsupervised learning, models learn from data without the need for the data to be labelled, the learning will try to discover the relationship and the hidden patterns between the different attributes. Unlike supervised learning, in which the model learns from input-output pairs, unsupervised learning builds a model without the need for having labeled data. Labeled data is considered to be time consuming and costly because it needs man power's efforts to be labeled, for this reason unsupervised learning is suitable for big data. In the health care sector, huge amounts of unlabeled data are generated automatically from medical records, imaging, and other resources. Unsupervised learning can help in many ways such as patient segmentation, medical image analysis, disease detection from medical records, symptom pattern recognition, and medications. Figure 1

includes the main unsupervised learning methods that will be discussed in the next subsections.

Unsupervised learning is used for clustering and for association rule mining, in clustering we have many concepts for clustering such as Density-based, Distribution-based, Hierarchical-based, and Centroid-based. The most common clustering algorithm is K-means which is considered centroid-based and suitable for small datasets. DBSCAN clustering algorithm (DBSCAN) stands for density-based spatial clustering of applications with noise, and it is suitable in outliers detection.

3.2.1 Clustering

Clustering is a technique that can be used to group similar data points together based on their characteristics. Thus, clustering, contrary to classification, does not rely on training vectors and is therefore particularly appropriate to tasks that try to find hidden structures of the data. The objective of clustering is to divide a set of data in a way that the data in a single cluster is most like the data in the other clusters in the same group compared to those in a different group. K-means, hierarchical clustering is some of the widely used types of clustering techniques also includes DBSCAN and Gaussian mixture models. Clustering has numerous uses, including, but not limited to customer segmentation from a marketing perspective; image compression; anomalies detection; and biological data analysis. This is perhaps because clustering can help to specify certain aspects of the data that can be used for decision making or else aid in the discovery of the data and find out, from this, the natural groupings which perhaps for some reasons, were not observed.

K-Means Clustering: K-Means Clustering is one of the most famous clustering techniques that belong to the unsupervised learning family of algorithms designed to categorize a given dataset into clusters on the basis of similarity of shared features. Thus, the goal of K-Means is to maximize the sum of squares within clusters and minimize the between cluster variation, so all the points belonging to one cluster are as close as possible to the same area in the space of features, and all the points of different clusters are as different as possible.

Hierarchical Clustering: Creates a tree-like structure of clusters through either agglomerative (bottom-up) or divisive (top-down) methods. It helps in understanding the hierarchy of clusters. The last is that hierarchical clustering does not presuppose the number of clusters to be created by a user. However, it makes a tree-like structure, in which the current clustering structure is nested, and offers a preview of how clusters are related across levels.

DBSCAN (Density-Based Spatial Clustering of Applications with Noise): Forms clusters based on dense regions of data points, making it useful for

identifying arbitrary-shaped. It is used for grouping the data on the basis of density of points in a particular dataset. It should be noted that DBSCAN is a kind of clustering algorithm which does not focus just on the discovery of different shaped clusters with differing sizes and figures, unlike such algorithms as K-Means, hierarchical clustering; particularly important for DBSCAN is a matter of noise, or let us say, of irregular points. This is especially useful for datasets that might not have spherical and clear cluster structures.

3.2.2 Association Rule Learning

Association Rule Learning is a type of supervised machine learning algorithm that is used to find hidden patterns amongst large sets of items. It is used most notably in market basket analysis, where it assists in the discovery of products that tend to occur together in transactions, and thus business can be able to act on its given understanding of customer buying habits as they form marketing strategies. Association rule mining is used in recommendation systems, basket market analysis, healthcare, and fraud detection. Its output is in if-then format that makes its decision making easy to interpret. We will cover two algorithms of association rule mining, Apriori algorithm and FP-Growth.

Apriori Algorithm: it is used to generate frequent item sets from the database and additionally, applies it for generating association rules. It is bottom up, that is, it first looks for one item set of frequency, then two item sets of frequency and so on, up to the required size. Frequent item sets do not have any items below the minimum support threshold; if they do, they are removed.

FP-Growth algorithm: The FP-Growth algorithm is an improvement over Apriori that avoids generating candidate item sets, which makes it more efficient, especially for large datasets. It builds a compact data structure called an FP-Tree (Frequent Pattern Tree) to represent frequent item sets, significantly reducing the time needed to find associations.

2.3 Dimensionality Reduction

Dimensionality reduction is one of the steps that a data preprocessing and feature selection in the machine learning and data analysis has to complete, where the number of inputs or attributes in the input data has to be limited, but the most powerful features must remain. It is most advantageous in cases where a huge number of features are being used in creating the model, this results in challenges such as overfitting, high computation time and difficulties in explaining the model. Dimensionality reduction is done so as to transform data and make it easier to handle while making models work well and also for visualization purposes retaining as much information of the dataset as possible.

Principal Component Analysis (PCA): PCA converts a high dimensional dataset into a lower dimensionality while at the same maximizing variability. PCA is particularly helpful when you work with datasets containing numerous

correlated features because in such cases it helps reduce complexity levels without compromise to data information content. PCA is one of the most commonly used techniques in exploration data analysis and its main objective is to minimize the dimensionality of a dataset. It achieves this by translating the variables in the originals into another set of values referred to as the principal components. Principal Components should be noted that the principal components are linear combinations of the initial characteristics, sorted by variance in descending order. The first principal component is given by direction along which gives the maximum variance of data while the second principal component gives the maximum variance perpendicular to the first principal component. These components are, thus, independent of each other and uncorrelated to each other.

3.3 Reinforcement learning

Reinforcement learning (RL) is one type of learning that contains artificial intelligence (AI) agents that interact with the environment in trial-and-error scenario to learn the optimal solution based on the rewards received from each option. Key concepts of RL contain agent, environment, state, action, and reward.

RL plays an important role in the healthcare domain in diabetes management to optimize the insulin dosing for patients (Daskalaki et al., 2013; Fox et al., 2020), also in chronic pain management by suggesting the suitable medication doses taking into account historical medical data, pain severity, and side effects, and in managing the hypertension disease by finding the optimal medication regimens based on patient profile (Shortreed et al., 2011).

RL algorithms can be categorized based on how they interact and learn from the environment and data. The most common ones are the model-free RL algorithms, the model-based RL algorithms, and the deep RL techniques.

3.3.1 Model-Free Reinforcement Learning Algorithms

Most model-free RL algorithms are those which map states to actions without modeling the environment. They are ideal for use where the behavior of the environment (response of the patient) is not fully understood or is too complicated to characterize in advance.

Q-learning: A Q-learning algorithm which is focused on estimating the action-value function (Q-function of state-action pairs for each of the values of the system states). self-improving techniques like Q-learning have been applied in adaptive diabetes management, where traces from the past are used to establish the right insulin doses (Fox et al., 2020).

Deep Q-Networks (DQN): A version of Q-learning which instead of utilizing a table to decide on the next best move uses artificial neural networks to estimate the Q-function for large or for any time, continuous states and actions spaces.

DQNs are particularly useful when dealing with large data of patients in which they have many features (Johnson, Gong, et al., 2017).

SARSA (State-Action-Reward-State-Action): An on-policy algorithm; somewhat similar to Q-learning, but which takes its Q-estimate update from the current policy in use. It is used when the training should ensure safety as the actions performed are closer to the learnt policy.

Policy Gradient Methods: These methods learn a policy directly by trying to maximize the expected cumulative reward by means of gradients heading in the right direction. For instance, REINFORCE formula is employed when updating the policy parameter in application areas that want to find the optimal personalized dosing schedules (Daskalaki et al., 2013).

3.3.2 Model-Based Reinforcement Learning Algorithms

Model-Based Reinforcement Learning is a kind of RL studies or uses a model of the environment as a part of the RL process. In reinforcement learning, an agent learns a policy to act in environment to achieve a cumulative reward more. Model based RL algorithms use an internal model of the environment which distinguishes it from model free RL algorithm that does not require this model. Model based RL involves the use of a model of what happens subsequently when an action is taken in a given state – commonly simulated by a transition model that just estimates the transition probabilities of the environment and a reward model that estimates the expected reward for an action-state pair. This model is then used to help to show and what needs to be done to maximize the greatest long-term rewards possible.

Dynamic Programming: Value Iteration VI and Policy Iteration PI methods are used in cases when the model of the environment is known, where we want to obtain the optimal policies by using iteration of the state and action values.

Dyna-Q: Reinforcement learning mixed with simulation where learning is made faster through the use of a model. This is helpful in patient-specific cancer therapy where creation of model of the tumor to the chemical has a benefit in the plan ((Yu et al., 2020).

3.3.3 Deep Reinforcement Learning Techniques

Deep RL can be brought as reinforcement learning coupled with high-capacity deep learning where an artificial agent accumulates sufficient experience to act adequately in reasonably complex surroundings. Deep RL is very effective for tasks, which have the big-sized and complicated state and/or action space such as video games, robotic control, and self-driving cars etc. Despite this, the Deep RL is similar to the traditional RL in that the functions that must be learned for learning and decision-making, like the value function or the policy, or the state-action value function (Q-value), are approximated by deep neural network. Both

techniques have been found to be very effective in solving problems that could not earlier be solved with RL methods.

One of the most effectual challenges of working with the large volume of patient data is the ability to manage the high dimensionality of the data in order to create a medication plan for highly specific patient sets which is a critical goal with the large volume and high dimensionality of patient information, records, genomics, imaging and other information sources. To address this problem, Deep Learning (DL) models are combined with Reinforcement Learning (RL), that allows for the development of complex systems capable of processing large-scale data and prescribing individualized logistic management plans.

Deep Deterministic Policy Gradient (DDPG): A method suitable for constant action space conditions; useful when actions (dosages) should be changed regularly.

Recurrent Neural Networks (RNNs): In applications where the patient's health state depends on historical data (e.g., chronic disease progression), **RNNs** are used to capture temporal dependencies in the data, allowing for better policy decisions.

These algorithms and techniques are applied based on the specific requirements of personalized medication management tasks, such as optimizing dosage, minimizing adverse effects, and considering long-term health outcomes. By leveraging these RL techniques, healthcare professionals can develop tailored interventions that improve the quality of care and patient outcomes while minimizing risks.

4. Data Sources for Machine Learning in Personalized Medicine

Medical data in health care has a diversity of sources and contents varying from structured, unstructured, and semi structured data. All these types are good resources for ML usage to help in classification and regression analysis. Electronic health records (EHRs) are used heavily in ML followed by images and genomic data, while the most recently used data is the data collected from wearable devices using remote monitoring technology. Biobanks and public health datasets contain important data related to biological samples and can be used as a data source for ML, also clinical trial data that includes drug info, side effects, patient response, and other detailed medical related information can be used as a data source as well. Figure 3 shows the mostly used data sources in the health domain.

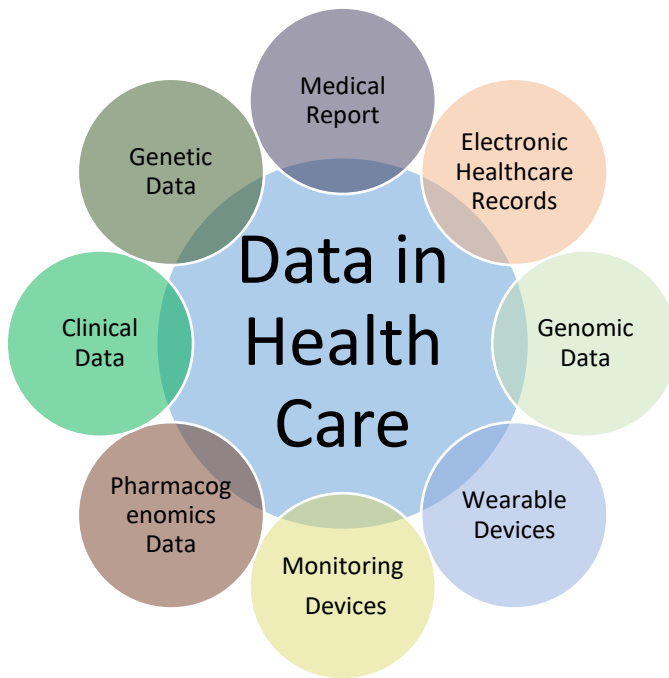


Figure 2. Data in Health Care

4.1 Electronic Health Records (EHRs)

The most popular data source in healthcare, EHRs include patient demographics, medical visits, diagnoses, test results, medications, and treatments. It is the digital version of all patients' papers and tests' results and radiology images. EHRs helps the health sector by sharing patient health related information easier and can improve the decision-making process and enhance patient outcomes.

Table1 shows that (Bertsimas et al., 2020; Bica et al., 2021; Ejike Innocent Nwankwo et al., 2024; Peng et al., 2021; Thakur et al., 2024; Yi et al., 2024) used EHRs in their studies, (Yi et al., 2024) used visits data, (Wang & Wang, 2023) used historical medication data, (Sarkar et al., 2023; Verma et al., 2019) used clinical data in their studies.

ERHs can include medical imaging and manage these images such as X-ray, CT scans, MRIs, and ultrasound images. HER systems integrate with archiving systems to access these images. Radiology reports and medical reports are considered to be part of the EHRs as well. (Gaur et al., 2022; Peng et al., 2021; Thakur et al., 2024) used images data in their studies.

4.2 Genetic and Genomic Data

Genetic and genomic data includes an individual's DNA, mutations, gene panel, and many other details that can help in discovering the high risk of individuals for certain disease based on their genetic map. It can also help in cancer therapies against tumors since they are genetic mutation. In pharmacogenomics, it can predict individual's response to certain medicine and risk factors.

Table1 shows that (Ejike Innocent Nwankwo et al., 2024; Gaur et al., 2022; Kaliappan et al., 2023; Thakur et al., 2024) used genetic and genomic data in their studies.

4.3 Wearables and Remote Monitoring Devices

Wearable devices are one of the emerged technologies in healthcare, contributing to personalized medications by having remote monitoring devices working 24/7 in collecting data from these wearables. This emerged technology can enhance chronic disease predication using the early collected diagnosis.

Wearable devices include smartwatches and fitness trackers, continuous glucose monitors (CGMs), blood pressure monitors, pulse oximeters, wearable biosensors, and smart clothing.

Some wearable devices are used in early detection of health issues, some are used for enhancing lifestyle by motivating people to walk more and to sleep better, some are used for rehabilitation care during the recovery process through monitoring vital signals and detect health deviations, some are used for remote patient monitoring in case of having limited mobility by using remote monitoring platforms, and some are used as assistant in deciding the optimal drug dosages.

Thomas Homescu, (2020) used wearable devices in their study while 19 used real-time monitoring data in their studies.

4.4 Pharmacogenomics Data

Pharmacogenomics is area of study that focuses on how genes can affect people responses to different drugs. It combines pharmacology and genomics to develop medications that are safe and effective based on person's genetic makeup. It includes genetic variants, drug transporter, and drug target variants data that can be publicly available such as PharmaGKB, or some datasets from research studies and biobanks, or from FDA's pharmacogenomics tables.

Pharmacogenomics can help in drug optimization, reducing adverse drug reactions (ADRs), cancer treatment, cardiovascular drugs, and neurology. This type of data is complex and has interactions between many genes, many variants that make the interpretation to be difficult. This type of data should be integrated with clinical practices and with EHRs efficiently.

(Malviya et al., 2023; Plant & Barton, 2021; Verma et al., 2019) used geonomics and pharmaceutical data in their studies.

5. Applications and Case Studies for Machine Learning in Treatment Optimization

This section highlights the transformative role of advanced algorithms in enhancing healthcare delivery by tailoring treatments to individual patient needs, improving decision-making, and maximizing treatment effectiveness across various medical conditions.

5.1 Drug Response Prediction

It is an important part of any personalized medicine planning, focusing on study and predicting how patients may interact with a certain medicine. The data included in this studied should combine between drugs efficiency, side effects, dosages, and safety from one side and genetic, biological, environmental factors from another side. Having this kind of data will leverage ML techniques to predict more accurate treatment and will reduce the expected side effects.

Drug response predictions factors are genetic factors, demographic factors, clinical factors, and microbiome influence. Genetic variations can significantly affect drug users and affects the efficiency, (Ingelman-Sundberg, 2004).

As an example of drug respond prediction, (Gage et al., 2008) used different support vector machine and regression tree machine learning algorithms for predicting the right dose of warfarin of each patient involve genetic factors which include CYP2C9 and VKORC1 and clinical factors like age of the patient and his/her weight.

5.2 Dosage Personalization

Drug dosage is a crucial aspect of personalized medicine, knowing the right amount of medicine targeted to a specific patient ensuring minimizing of side effects is a big challenge that ML can take part in. Regression models such as linear regression and random forests are used to predict suitable drug dosage based on many features including the demographic and the medical history (Gage et al., 2008). Support Vector Regression (SVR) and Gradient Boosting Machines (GBM) can also predict the right dosage by finding the match between patient characteristics and dosage levels (Shortreed et al., 2011).

As an example of dosage personalization, (Daskalaki et al., 2013) used the reinforcement learning and SVM to find the optimal insulin dose for diabetic patients, while (Zhu et al., 2020) proposed an insulin bolus advisor for type1 diabetes patients using deep reinforcement learning.

5.3 Choosing the Best Treatment Option

Analyzing heavy data such as genetic, demographic, clinical, and lifestyle using machine learning models can give recommendations about the most suitable treatment option for a specific patient. These models can maximize treatment efficiency and decrease side effects. Random Forest and Gradient Boosting Machine are applied to make prediction on treatment results based on age, genetic makeup and disease nature. They can suggest the most efficient treatment by using algorithms on similar case-studies used by the system (Kourou et al., 2015). In (Esteva et al., 2019) deep neural networks are applied by analyzing large datasets including genomic sequences, medical images and clinical notes to make predictions of treatment outcomes. These models can find complex relationships between features that allow presenting the treatment recommendation in oncology.

As an example, to choose the best treatment option, a group of researchers from Stanford University used reinforcement learning to develop a personalized treatment for sepsis patients and their model was able to dynamically learn the best treatment based on patient's conditions, (Komorowski et al., 2018). The used data set contains around 20,000 sepsis patients that include patient demographics, lab results, treatments, and clinical notes.

6. Challenges and Limitations of Using ML in Personalized Treatment

Using ML in personalized treatment has many advantages but also facing many challenges and limitations. Here are some key challenges:

6.1 Data complexity and quality

Data collected from health care sector is complex and diverse; complexity comes from having different formats of data and diversity comes from the fact that each data type is collected from different sources that often needs to be integrated together under one platform. Data integration faces by itself a big challenge due to data consistency and completeness, missing values for example should be resolved before using data, otherwise model performance will be affected.

Major issues of data complexity and quality include high dimensional data, noisy data, data cleaning, preprocessing, biased data, and incomplete data. There should be data governance framework of policies, standards, processes, and tools that organizations should follow to ensure data quality and consistency.

6.2 Data Privacy Concerns

Patient data is highly sensitive, using ML in personalized medicine means that ML techniques will deal with high amounts of personal information. Data privacy should be protected, and it should be guaranteed by using some regulations such

as HIPPPA and GDPR. There should be a balance between improving treatment and between maintaining confidentiality.

Large amounts of data are collected from different sources, such as EHRs and wearable devices, this represents a challenge since this data needs to be integrated and during this process the risk of unauthorized access will be significant. For this reason, some data protection measures should be applied to hide patient identity such as data anonymization and differential privacy.

6.3 Ethical Concerns

To ensure that the decision made by the ML systems is ethical and understandable, ethical standards must be set for the purpose of ascertaining justification for the decision made. The problem has been foreseen with conventional ML models that are sensitive to the training data sets used. If the datasets are not sufficiently diverse and do not include most of the cases, the recommendation and decisions will be biased. The doctors and practitioners should be more involved in the decision made by the ML models to ensure the ethical perspective has been upheld appropriately and there is no ethical issue as per the doctors and practitioners. Data ownership is a major issue as well; patients must be made aware of how information relating to them is being used and if any third party disclosed patient's data without consent which may lead to legal action.

6.4 Model Interpretability

Interpretability of a model seeks to explain how the model arrived at its decision. This argument is one of the primary obstacles to building confidence, openness, and accountability in healthcare areas for both clinicians and patients. Some ML models such as deep learning in particular are not easily interpretable compared with other models that have a sequence of understandable steps such as decision trees and rule-based models.

In order to meet this challenge one approach is the creation of models with interpretability capacity to provide more transparent decision-making processes. In order to close this gap researchers have been working on techniques like Local Interpretable Model-agnostic Explanations (LIME) or SHapley Additive exPlanations (SHAP) with the intention of making the model outputs comprehensible. For example, LIME explains predictions based on discrete local models that are constructed around predictions; while clinicians may not fully understand the larger model, LIME gives them enough understanding of why a certain prediction was made.

7. Emerging Trends and the Future of ML in Personalized Medicine

Novel trends in machine learning (ML) are making significant impacts in how the future of personalized medicine will look like from being precise, efficient

and patient centered. Recent developments in techniques including deep learning, genomic data analysis and multi-modal are helping in creating different treatment models for patients. Some of these trends are Omics data with AI, early disease biomarkers with predictive analytics, and reinforcement learning for adaptive treatment regimens are transforming diagnosis, management, and treatment of diseases. Omics can be described as an umbrella term for a set of technologies and or disciplines that undertake the systematic study of biological parts such as genes, proteins, RNA, metabolites and microbe populations in the biological system or organism in question.

Further, due to privacy issues, Federated Learning is also emerging to permit model training with patient records update but not sharing its information. The near future of replacing existing clinical applications through ML in PM as well as its potential as a tool for improving existing clinical applications is promising and will facilitate the early, timely, and patient-specific delivery of health care to achieve the best therapeutic effects at lower costs.

7.1 Explainable AI (XAI)

Explainable AI (XAI) is a major trend of XAI focuses on explaining the ‘black box’ of many machine learning models, especially in medicine. Popular techniques like deep learning are not transparent, which is why more traditional methods in the ML field are viewed negatively. They have observed that such a lack of explainability has posed a challenge to healthcare professionals to understand how and why a certain decision or prediction has been made when such a decision involves the care of patients. One of the biggest benefits of XAI is its relevance, which is simply in offering explanations that can be well understood in the field of healthcare. In this regard, some of the most typical ones include SHAP (SHapley Additive exPlanations) and LIME (Local Interpretable Model-agnostic Explanations). These methods provide better means to visualize and explain the effect of several input attributes in determining the output and help to fill the gap between sophisticated models and practical medical care decisions.

The interpretation offered by various XAI techniques not only makes enhancement of regular ML models in clinical practice possible but is also used for compliance with ethical and regulatory requirements. Because the AI-based decisions found in healthcare result in explicit methodologies for the patient’s treatment plan, it is essential to emphasize the accountability of AI-based decision making. Moreover, performance enhancements are therapeutically annexed to XAI as well in which researchers can find preconceptions or false associations and get to know which feature is dominating the decisions of the model and, therefore, improve the model and its reliability. For instance, if a model makes a prognosis towards increased heart disease risk, XAI can explain whether this is due to lifestyle choices or genetic predisposition, or perhaps incorrect entries in data.

The ability to criticize model output will increase the trustworthy model and the AI reliability in healthcare.

7.2 Federated Learning

Federated learning (FL) is a new technology used to address the privacy issue in personalized medicine. FL is an emerging branch of artificial intelligence and ML (W. Yang, Zhang, Ye, et al., 2019), it is a distributed approach in which its main component is nodes; these nodes contain data and sometimes are called edge nodes. These nodes can learn directly from the data they contain, and the model transfers its output to a central node (server) that receives output from other nodes and shares the results with other participants. FL is considered as a collaborative, decentralized privacy-preserving method to attach the challenges of storing data and data privacy (Li et al., 2020).

The advantages of FL are especially apparent in the rare disease investigation and large-scale clinic trials, where datasets from individual institutions are inadequate to develop an accurate ML model. Through aggregating the information obtained from the data distributed in several institutions, FL can contribute to constructing more accurate models that can serve more patients. For example, when developing federated models for prognosis of disease advancement, it can be effective when training of the model incorporates the patient data obtained from different regions, in an attempt to simplify the model's applicability on a wider populace. However, integration between hospitals, research centers, and pharmaceutical companies becomes more viable since the privacy dilemma is reduced greatly hence fast exchange of information.

7.3 AI-Driven Drug Discovery

Artificial intelligence continues to be a game changer in translating possibilities in the field of drug development and customized treatment by making drug discovery faster and more efficient. The typical method of drug discovery may take about a decade depending on the process of discovery, development and all entail costs. Approaches based on artificial intelligence and deep learning decrease the time spent on the search for prospective targets and therapeutic agents, with the help of large amounts of biomedical data and chemical databases. AI-driven drug discovery can lead to the identification of drugs that are tailored to specific subgroups of patients, thus increasing the likelihood of treatment efficacy (Zhavoronkov et al., 2019).

A successful application of AI in drug discovery is the development of personalized cancer therapies. Deep learning algorithms have been used to browse patient's DNA and determine which of the mutations are causing their cancer. For instance, some companies such as BenevolentAI and Insilico Medicine have been applying AI to discover drugs and reinvent existing drugs for the management of particular cancer types. These AI-based techniques are

also used in forecasting the probability of side effects and toxicity levels which assist the identification of probable side effects in the early developmental phases thus enhancing the general efficiency of drug candidates. This approach has become especially valuable during the COVID-19 outbreak when AI models were used to predict the efficiency of existing drugs for the treatment of the SARS-CoV-2 virus. Therefore, AI-driven drug discovery represents a significant step towards making personalized medicine more accessible and efficient(Vamathevan et al., 2019) .

7.4 Integration with Telemedicine

When considering general trends in personalized medicine, telemedicine together with ML is becoming a powerful trend. Telemedicine has been identified as a rapidly growing concept due to patients' and healthcare providers' growing use of teleconsultations and remote patient monitoring, particularly after the COVID-19 crisis. Such remote service as telemedicine integrated with the ML models allow analyzing the patient's information in real-time and recommends personalized therapy. When incorporated with telehealth, the efficiency of ML algorithms is demonstrated in factors like pulse rate, blood sugar, and respiratory rates and detects the deviations. His continuous monitoring can prevent complications, promote timely intervention, and deliver personalized treatment plans tailored to the unique needs of each patient (Ravi et al., 2016).

Proactive health management is another advantage of this integration because it constantly monitors health state and can intervene immediately. For instance, smartwatches and continuous glucose monitors CGM deliver a high volume of data, and an accurate ML model can present relevant information helping both the patient to modify behavior and the clinician to diagnose the disease. In patients with chronic illnesses such as diabetes, an ML model can determine the likelihood of hyperglycemic or hypoglycemic episodes and the patient can act in advance. This change transfers the conventional model of care from a stretcher based or acute care model to a chronic care model that is proactive and individualized to the development in the patient's status (He et al., 2019).

8. Conclusion

ML as a subdiscipline of AI has radically enhanced personalized medication management to improve the decision-making process of care providers. ML models can estimate a patient's outcomes when given specific treatments by using other data sources such as EHRs, genetic data, and Realtime data from wearables among others. The trial-and-error way of handling medications is eliminated, and patients gain access to quicker treatment by individual health plans. Supervised learning algorithms, including decision trees and the support vectors method, have been applied to the enhancement of drug-response predictions and proposals of individual dosages of the drugs. Moreover,

application of ML helps in biomarkers and patients' subpopulation discovery, who could benefit from particular therapies. It's important for such illnesses as cancer and autoimmune; when it comes to medication, nothing is better than tailored programs and action. Most of the unsupervised learning have been employed to detect the underlying structure within patient data sets in order to categorize patients into different treatment groups. This means there is a timely prescription of treatments that give optimum therapeutic success with least side effects.

The most transformational application that has been used in the decision support system where ML helps clinicians to integrate prediction into the treatment plan. Also, reinforcement learning has been used to adapt intervention strategies to patient improvement, which means that care is constantly refined. This solves a major problem for healthcare providers and makes patient care much safer for the patients themselves by offering immediate notification and advice. ML has been confirmed to be a valuable resource in developing the field of personalized medicine, enhancing patient experiences while getting the best results and using resources in healthcare efficiently. Looking Forward Machine learning in healthcare has a bright prospect and is only going to become better with the integration of explainable AI (XAI). A major issue of ML currently in healthcare is the inability to explain decisions made especially by opaque systems such as deep learning ones. AI for Explainability aims to aid these models to be more interpretable to a clinician so the thought processes behind suggested treatments are apparent. It will help create trust and enhance the relationship between health care professionals and technology. Continued work on XAI will serve as a key method for moving the ML deeper into typical clinical work since it directly tackles the issue of applied technology.

With increased application of AI in drug discovery, personalized treatment procedures have also been poised for reform in the coming future. Artificial intelligence is applied to evaluate chemical libraries and predict drug-target interactions, tenfold compared to the previous methods to define new personalized therapies. By integrating AI with Genomic and patient-sampled data, the future is set for better drugs with less risk on the patient. The utilization of ML in telemedicine and home monitoring will continue to promote precision in treatment making health care more patient centered and not location sensitive. Call to Action For this approach of using ML to achieve the best results in patient care to be realized, there must be close cooperation between healthcare providers, researchers, technologists and policy makers. The clinicians offer domain knowledge so that they understand the clinical environment, while the data scientists and technologists offer technical knowledge to build sound models. Interdisciplinary cooperation will therefore guarantee that solutions created using machine learning are not merely scientifically correct but are also clinically applicable and helpful to patients. Successful implementation of ML into the

healthcare niche calls for collaborative efforts to design implementable tools that work efficiently and seamlessly in the workflows that are already present in this sector.

There is therefore also a need for ethics and rationale in ML when they are connecting health care systems and developing them. There is the need to set standards that should be adhered to in order to protect the privacy of patients, as well as to ensure the application of accountability by the models and provide equal access to the technologies by the policymakers. To ensure the efficacy and the permissiveness and non-maliciousness of ML healthcare system, guidelines should be made for data gathering and distribution, as well as model assessment. Moreover, guidelines in ethics should be provided in order to avoid discriminative functions of the ML models that directly impact the effectiveness of the treatment. This can only be done when all parties work hand in hand in developing models that are in today's society perceived as completely fair and non-bias and fully accepted by other personnel in the medical field.

Finally, there is a strong need for educational efforts to address the requirement to develop and implement more educational attainment with healthcare professionals who work in the near future where the concept of ML has become prevalent and integrated into the patient care service delivery system of healthcare organizations. Appropriate clinical training, various workshops, and interdisciplinary education can predispose clinicians to understanding the essence of the ML models as well as to implementing them in practice. Training young as well as experienced healthcare professionals on AI will guarantee that they are ready to enhance the use of ML towards the enhancement of healthcare provision thus better patient care. The practitioners in this field should also be motivated to include the end-users in their practice for the enhanced overall understanding of their requirements to help improve the existing and creating innovative and highly effective and efficient healthcare ML tools.

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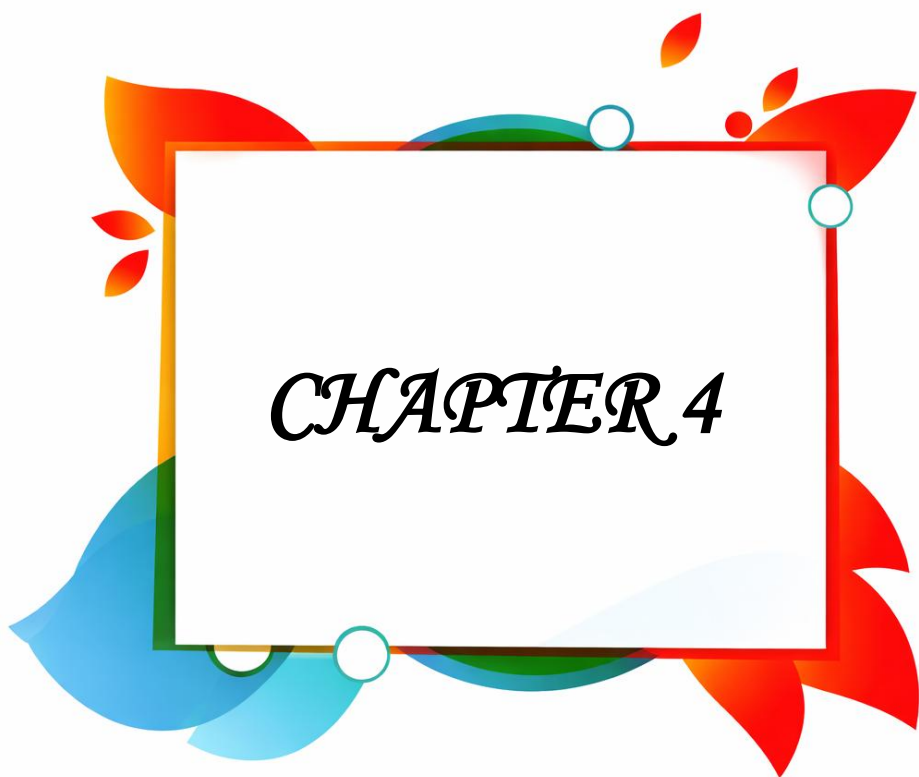
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Antifungal Resistance and Emerging Therapeutic Strategies in Oral Candidiasis

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1. Introduction

Oral fungal infections have gained increasing importance in modern dental and medical practice, particularly with the growing number of immunocompromised and medically complex patients. Among these infections, oral candidiasis remains the most frequently encountered opportunistic fungal disease of the oral cavity and continues to represent a substantial diagnostic and therapeutic challenge for clinicians worldwide (Akpan & Morgan, 2002). Although *Candida* species are considered normal commensal microorganisms within the oral ecosystem, alterations in host immunity, oral microbial balance, salivary composition, or mucosal integrity may transform these organisms into invasive pathogens capable of causing persistent and recurrent disease.

In recent decades, the epidemiology of oral candidiasis has changed considerably. Improvements in cancer therapy, organ transplantation, intensive care medicine, and immunosuppressive treatment protocols have significantly increased the number of susceptible individuals. In parallel, prolonged survival of medically compromised patients has contributed to a marked rise in opportunistic fungal infections (Perlin et al., 2017). Additionally, the widespread use of broad-spectrum antibiotics and corticosteroids has facilitated disruption of oral microbial homeostasis, allowing fungal overgrowth and biofilm-associated infections.

Historically, *Candida albicans* has been the dominant species isolated from oral candidiasis lesions. However, epidemiological investigations performed over the last two decades have demonstrated a growing prevalence of non-*albicans* *Candida* species including *Candida glabrata*, *Candida tropicalis*, *Candida krusei*, and the emerging multidrug-resistant pathogen *Candida auris* (Rodrigues et al., 2014). These organisms are clinically important because many demonstrate intrinsic or acquired resistance to commonly prescribed antifungal agents.

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Antifungal resistance has therefore become one of the most important contemporary concerns in oral medicine and infectious disease management. Resistance mechanisms are multifactorial and include efflux pump activation, mutations affecting antifungal target enzymes, altered membrane sterol composition, stress response adaptation, and, most importantly, biofilm formation (Cowen et al., 2015). Biofilm-associated fungal cells may exhibit resistance levels dramatically higher than their planktonic counterparts, making conventional therapies increasingly ineffective.

Conventional treatment approaches such as topical nystatin and systemic azoles remain fundamental components of oral candidiasis management. Nevertheless, limitations including drug toxicity, reduced patient compliance, frequent recurrence, resistance development, and inadequate biofilm penetration have stimulated intensive research into alternative therapeutic approaches. Consequently, major advances have been made in antifungal drug delivery systems, nanotechnology, phytotherapy, microbiome modulation, photodynamic therapy, and immunotherapy.

The purpose of this chapter is to provide a detailed and contemporary overview of oral candidiasis with special emphasis on antifungal resistance and emerging therapeutic strategies. Current concepts regarding epidemiology, pathogenesis, fungal virulence, biofilm biology, conventional antifungal therapy, resistance mechanisms, and novel treatment modalities are comprehensively reviewed and critically discussed.

1.1 Historical Perspective of Oral Candidiasis

Descriptions resembling oral candidiasis can be traced back to ancient medical writings. However, the fungal etiology of the disease was not recognized until the nineteenth century following advances in microbiology and microscopy. Early studies identified *Candida albicans* as the principal etiologic organism responsible for thrush and other mucosal fungal infections.

During the twentieth century, development of polyene antifungals such as nystatin and amphotericin B represented major milestones in antifungal therapy. The subsequent introduction of azole antifungals, particularly fluconazole, revolutionized management of fungal infections due to improved oral bioavailability and reduced toxicity.

Despite these therapeutic advances, widespread antifungal use has resulted in increasing resistance and emergence of difficult-to-treat fungal species. Consequently, oral candidiasis has evolved from a relatively straightforward opportunistic infection into a more complex disease entity requiring multidisciplinary management strategies.

1.2 Clinical Importance in Dentistry

Oral candidiasis is highly relevant to multiple dental specialties including oral medicine, oral and maxillofacial surgery, prosthodontics, orthodontics, periodontology, pediatric dentistry, and geriatric dentistry. Denture-related fungal infections remain among the most common complications associated with removable prostheses. Similarly, xerostomia-associated candidiasis is frequently encountered in oncology patients receiving radiotherapy for head and neck malignancies.

In oral surgery practice, fungal infections may complicate postoperative wound healing, implant therapy, sinus augmentation procedures, and management of medically compromised individuals. Moreover, fungal biofilms on implant surfaces have become increasingly important in peri-implant disease research.

The relationship between oral candidiasis and systemic diseases further highlights the importance of early diagnosis and effective management. In some cases, oral candidiasis may represent the first clinical manifestation of underlying immunodeficiency or systemic disease.

1.3 Scope of the Chapter

This chapter aims to provide a clinically oriented and evidence-based review of oral candidiasis and antifungal resistance. Particular emphasis is placed on:

- fungal virulence mechanisms,
- biofilm-associated resistance,
- non-*albicans Candida* infections,
- diagnostic advances,
- antifungal susceptibility,
- nanoparticle-based drug delivery systems,
- photodynamic therapy,
- probiotics and microbiome modulation,
- phytotherapeutic compounds,
- immunotherapeutic approaches,
- and future perspectives in antifungal management.

The chapter also discusses translational and clinical implications of emerging therapeutic approaches that may contribute to overcoming antifungal resistance in oral fungal infections.

2. Epidemiology of Oral Candidiasis

Oral candidiasis represents one of the most common fungal infections encountered in oral medicine and dental practice. The condition develops as a consequence of opportunistic overgrowth of *Candida* species within the oral cavity and is strongly associated with disruption of local or systemic host defense mechanisms (Akpan & Morgan, 2002; Williams & Lewis, 2011). Although *Candida* species are part of the normal oral microbiota in many healthy individuals, changes in the oral environment may facilitate fungal transition from harmless commensal organisms to pathogenic invasive microorganisms.

The incidence of oral candidiasis has increased considerably during recent decades. This increase is closely associated with aging populations, widespread use of immunosuppressive therapies, prolonged antibiotic exposure, increased prevalence of diabetes mellitus, advances in cancer therapy, and growing numbers of medically compromised patients (Perlin, Rautemaa-Richardson, & Alastruey-Izquierdo, 2017). Furthermore, oral fungal infections have become increasingly important in intensive care medicine, oncology, hematology, transplantation, and geriatric healthcare settings.

Historically, *Candida albicans* has been recognized as the primary causative organism of oral candidiasis. However, recent epidemiological studies have demonstrated a growing prevalence of non-*albicans* *Candida* species such as *Candida glabrata*, *Candida tropicalis*, *Candida krusei*, and *Candida auris* (Rodrigues, Silva, & Henriques, 2014). These species are clinically significant because many exhibit intrinsic or acquired resistance to commonly used antifungal agents.

The increasing emergence of antifungal resistance has become a major global healthcare concern. Resistance mechanisms including biofilm formation, efflux pump overexpression, altered ergosterol synthesis, target gene mutations, stress response pathways, and phenotypic adaptation substantially compromise therapeutic efficacy (Cowen et al., 2015). Consequently, recurrent infections, chronic disease persistence, and treatment failures have become more common.

Conventional therapies including nystatin, fluconazole, ketoconazole, and amphotericin B remain widely used in clinical practice. Nevertheless, concerns regarding drug toxicity, resistance development, biofilm-associated infections, and limited efficacy against resistant isolates have stimulated intensive research into alternative therapeutic strategies.

Recent advances in molecular microbiology, nanotechnology, biomaterials, microbiome research, and immunotherapy have opened new avenues for antifungal treatment. Novel therapeutic approaches include photodynamic therapy, nanoparticles, probiotics, antimicrobial peptides, phytotherapeutic compounds, quorum sensing inhibitors, biofilm-targeted therapies, and synergistic combination regimens (Rautemaa & Ramage, 2011).

This chapter provides a comprehensive overview of oral candidiasis with particular emphasis on antifungal resistance mechanisms and emerging therapeutic strategies. Current evidence regarding pathogenesis, biofilm biology, diagnosis, conventional treatment modalities, and innovative therapies is critically discussed.

2. Epidemiology of Oral Candidiasis

2.1 Global Prevalence

Oral candidiasis affects millions of individuals worldwide and remains one of the most prevalent fungal diseases involving the oral cavity. Oral colonization by *Candida* species occurs in approximately 30–60% of healthy adults, whereas colonization rates may exceed 80% in immunocompromised individuals (Sardi et al., 2013). The prevalence varies depending on geographic location, age, systemic health status, oral hygiene habits, denture use, and diagnostic criteria.

Neonates, elderly patients, denture wearers, and individuals with impaired immune function represent high-risk groups. In HIV-positive individuals, oral candidiasis was historically among the earliest and most common opportunistic infections before the widespread implementation of antiretroviral therapy (Patton et al., 2009).

2.2 Predisposing Factors

Local Factors

Several local environmental changes facilitate fungal colonization and pathogenic transformation:

- Poor oral hygiene
- Denture wearing
- Xerostomia
- Smoking
- Orthodontic appliances
- High carbohydrate intake

- Salivary gland dysfunction
- Mucosal trauma
- Reduced salivary antimicrobial activity

Systemic Factors

Systemic conditions substantially increase susceptibility to oral candidiasis:

- Diabetes mellitus
- HIV/AIDS
- Leukemia
- Chemotherapy
- Radiotherapy
- Organ transplantation
- Corticosteroid therapy
- Broad-spectrum antibiotic use
- Hematological malignancies
- Nutritional deficiencies
- Advanced age

2.3 Epidemiological Shift Toward Non-albicans Species

Recent epidemiological studies indicate an increasing prevalence of non-*albicans* *Candida* species. This shift is clinically important because many of these species demonstrate reduced susceptibility or intrinsic resistance to conventional antifungal agents (Silva et al., 2012).

Table 1. Clinically Important *Candida* Species in Oral Candidiasis

Species	Clinical Characteristics	Resistance Profile
<i>Candida albicans</i>	Most common pathogen	Increasing azole resistance
<i>Candida glabrata</i>	Frequent in elderly/immunocompromised	Reduced azole susceptibility
<i>Candida tropicalis</i>	Common in neutropenic patients	Multidrug resistance reported
<i>Candida krusei</i>	Opportunistic pathogen	Intrinsic fluconazole resistance
<i>Candida auris</i>	Emerging nosocomial pathogen	Frequent multidrug resistance

3. Oral Microbiome and Candida Ecology

The oral cavity hosts one of the most diverse microbial ecosystems in the human body. The oral microbiome includes bacteria, fungi, viruses, protozoa, and archaea interacting within a complex ecological network.

Fungi constitute an important but often underestimated component of the oral microbiome. The term “oral mycobiome” refers to fungal communities inhabiting the oral cavity. Under physiological conditions, fungal growth is tightly regulated by host immunity, saliva, bacterial competition, and mucosal barriers.

Disruption of microbial homeostasis leads to dysbiosis and facilitates fungal overgrowth. Antibiotic therapy represents one of the most important causes of oral dysbiosis because elimination of bacterial competitors allows uncontrolled fungal proliferation.

3.1 Candida-Bacterial Interactions

Interactions between *Candida* species and oral bacteria significantly influence biofilm formation and virulence.

Examples include:

- *Candida albicans* + *Streptococcus mutans*
- *Candida albicans* + *Staphylococcus aureus*
- *Candida albicans* + *Porphyromonas gingivalis*

These synergistic interactions enhance:

- Biofilm maturation
- Antimicrobial resistance
- Tissue invasion
- Quorum sensing signaling
- Immune evasion

3.2 Saliva and Host Defense

Saliva plays a central role in controlling fungal colonization through:

- Mechanical cleansing
- Antimicrobial peptides
- Histatins
- Lactoferrin

- Lysozyme
- Secretory IgA

Reduced salivary flow substantially increases susceptibility to oral candidiasis.

4. Pathogenesis of Oral Candidiasis

4.1 Adhesion Mechanisms

Adhesion to epithelial surfaces is the initial step in fungal colonization.

Key fungal adhesins include:

- Agglutinin-like sequence proteins (Als family)
- Hyphal wall protein 1 (Hwp1)
- Integrin-like molecules

These molecules facilitate attachment to:

- Oral epithelial cells
- Salivary glycoproteins
- Denture acrylic surfaces
- Orthodontic materials
- Implant surfaces

4.2 Morphological Transition

One of the most important virulence factors of *Candida albicans* is its ability to transition between yeast and hyphal forms.

Hyphal transformation contributes to:

- Tissue invasion
- Enhanced adhesion
- Biofilm maturation
- Immune escape

4.3 Biofilm Formation

Biofilm formation represents one of the most important pathogenic mechanisms in oral candidiasis.

Stages of Biofilm Formation

1. Initial adhesion
2. Intermediate proliferation
3. Biofilm maturation
4. Dispersion

Biofilms are highly resistant microbial communities embedded within extracellular polymeric matrices.

Clinical Importance of Biofilms

Biofilm-associated infections are characterized by:

- Increased antifungal resistance
- Chronic persistence
- Recurrent infections
- Reduced drug penetration
- Presence of persister cells

Biofilm-associated fungal cells may demonstrate resistance levels up to 1000-fold higher than planktonic cells (Ramage et al., 2009).

4.4 Hydrolytic Enzymes

Candida species produce extracellular enzymes that facilitate tissue destruction and invasion.

These include:

- Secreted aspartyl proteinases (SAPs)
- Phospholipases
- Lipases
- Hemolysins

5. Clinical Manifestations

5.1 Pseudomembranous Candidiasis

Pseudomembranous candidiasis, commonly known as thrush, presents clinically as white removable plaques covering erythematous mucosa.

5.2 Erythematous Candidiasis

This form is characterized by painful red mucosal lesions and frequently occurs following antibiotic therapy.

5.3 Hyperplastic Candidiasis

Chronic hyperplastic candidiasis presents as non-removable white plaques and may exhibit epithelial dysplasia.

5.4 Denture Stomatitis

Denture stomatitis is strongly associated with denture biofilms and poor prosthesis hygiene.

Newton Classification

- Type I: Localized inflammation
- Type II: Generalized erythema
- Type III: Granular hyperplasia

5.5 Angular Cheilitis

Angular cheilitis commonly involves mixed fungal-bacterial infections and presents with painful fissures at the lip commissures.

6. Diagnosis of Oral Candidiasis

6.1 Clinical Diagnosis

Clinical examination remains fundamental in diagnosis.

6.2 Cytological and Histopathological Evaluation

Diagnostic staining methods include:

- Periodic acid-Schiff (PAS)
- Grocott methenamine silver staining

6.3 Microbiological Culture

Common culture media:

- Sabouraud dextrose agar
- CHROMagar

6.4 Molecular Diagnostics

Advanced molecular techniques include:

- Polymerase chain reaction (PCR)
- Quantitative PCR
- MALDI-TOF mass spectrometry

- Next-generation sequencing

6.5 Antifungal Susceptibility Testing

Antifungal susceptibility testing has become increasingly important due to growing resistance.

Methods include:

- Broth microdilution
- Disk diffusion
- E-test

7. Conventional Antifungal Therapy

7.1 Polyenes

Nystatin

Nystatin remains one of the most widely prescribed topical antifungal agents in dentistry.

Mechanism:

- Ergosterol binding
- Membrane disruption
- Cellular leakage

Advantages:

- Minimal systemic absorption
- Relatively safe

Limitations:

- Frequent dosing
- Poor compliance
- Limited biofilm efficacy

Amphotericin B

Amphotericin B demonstrates broad-spectrum antifungal activity but is associated with nephrotoxicity.

7.2 Azoles

Fluconazole

Fluconazole inhibits ergosterol biosynthesis by targeting lanosterol 14 α -demethylase.

Advantages:

- High oral bioavailability
- Favorable pharmacokinetics

Limitations:

- Drug resistance
- Drug interactions
- Hepatotoxicity risk

Other Azoles

- Ketoconazole
- Itraconazole
- Voriconazole
- Posaconazole

7.3 Echinocandins

Echinocandins inhibit β -(1,3)-D-glucan synthesis.

Examples:

- Caspofungin
- Micafungin
- Anidulafungin

8. Mechanisms of Antifungal Resistance

8.1 Efflux Pump Overexpression

Efflux pumps actively remove antifungal drugs from fungal cells.

Important resistance genes:

- CDR1
- CDR2
- MDR1

8.2 Target Enzyme Mutations

Mutations in ERG11 reduce azole binding affinity.

8.3 Altered Ergosterol Biosynthesis

Alterations in membrane sterol composition reduce antifungal susceptibility.

8.4 Biofilm-Mediated Resistance

Biofilms contribute to resistance through:

- Reduced drug penetration
- Extracellular matrix protection
- Persister cells
- Metabolic heterogeneity

8.5 Stress Response Pathways

Stress adaptation pathways including calcineurin and heat shock proteins contribute to fungal tolerance.

Table 2. Major Mechanisms of Antifungal Resistance

Resistance Mechanism	Clinical Consequence
Efflux pump activation	Reduced intracellular drug concentration
ERG11 mutations	Azole resistance
Biofilm formation	Persistent infection
Stress response activation	Drug tolerance
Altered membrane sterols	Reduced antifungal susceptibility

9. Emerging Therapeutic Strategies

The limitations of conventional antifungal agents and the rapid emergence of resistant fungal strains have accelerated the search for alternative and adjunctive therapeutic approaches. In recent years, significant advances have been achieved in molecular microbiology, biomaterials, nanotechnology, and immunology, leading to development of novel antifungal strategies targeting fungal virulence, biofilm formation, microbial communication systems, and host immune responses.

Unlike traditional antifungal agents that primarily target fungal membrane sterols or cell wall synthesis, many emerging therapies aim to interfere with multiple pathogenic pathways simultaneously. This multifactorial approach may reduce resistance development and improve therapeutic efficacy.

9.1 Natural Products and Phytotherapeutics

Naturally derived compounds have attracted substantial attention because of their broad biological activities, relatively low toxicity, and potential synergistic interactions with conventional antifungal agents.

Medicinal plants have historically been used in treatment of fungal infections in traditional medicine systems worldwide. Modern pharmacological research has confirmed that numerous phytochemicals exhibit antifungal, antioxidant, anti-inflammatory, and anti-biofilm properties.

Thymoquinone

Thymoquinone (TQ), the principal bioactive component of *Nigella sativa* (black seed), has emerged as one of the most promising phytotherapeutic antifungal agents.

Reported antifungal mechanisms of thymoquinone include:

- disruption of fungal cell membrane integrity,
- induction of oxidative stress,
- mitochondrial dysfunction,
- inhibition of fungal biofilm formation,
- suppression of virulence-related genes,
- and synergistic interaction with conventional antifungal drugs.

Experimental studies have demonstrated antifungal activity of thymoquinone against *Candida albicans*, *Candida glabrata*, *Candida tropicalis*, and *Candida krusei*. In addition to direct antifungal effects, thymoquinone possesses anti-inflammatory and antioxidant properties that may contribute positively to mucosal healing.

Importantly, synergistic interaction between thymoquinone and nystatin has been demonstrated in oral candidiasis isolates, suggesting potential for combination therapy approaches aimed at reducing antifungal resistance and improving therapeutic outcomes (Özdal Zincir et al., 2022).

Curcumin

Curcumin, a polyphenolic compound derived from *Curcuma longa*, exhibits broad-spectrum biological activity including antifungal, antioxidant, anti-inflammatory, and anticancer effects.

Mechanisms contributing to antifungal activity include:

- membrane destabilization,
- reactive oxygen species generation,
- inhibition of fungal adhesion,
- biofilm suppression,
- and interference with fungal signaling pathways.

Curcumin has also attracted considerable interest as a photosensitizer in photodynamic therapy protocols.

Essential Oils

Essential oils contain multiple bioactive compounds capable of disrupting fungal membranes and inhibiting fungal growth.

Commonly investigated essential oils include:

- tea tree oil,
- clove oil,
- thyme oil,
- oregano oil,
- peppermint oil,
- and cinnamon oil.

Many essential oils exhibit strong anti-biofilm activity and may enhance susceptibility of resistant fungal strains to conventional antifungal agents.

Table 3. Selected Natural Compounds with Reported Antifungal Activity

Compound	Main Source	Proposed Mechanisms
Thymoquinone	<i>Nigella sativa</i>	Membrane disruption, oxidative stress
Curcumin	<i>Curcuma longa</i>	Biofilm inhibition, ROS generation
Eugenol	Clove oil	Cell membrane damage
Cinnamaldehyde	Cinnamon oil	Ergosterol interference
Carvacrol	Oregano oil	Membrane permeability changes

9.2 Antimicrobial Peptides

Antimicrobial peptides (AMPs) are naturally occurring molecules involved in innate immune defense systems. These peptides exhibit broad-spectrum antimicrobial activity against bacteria, fungi, and viruses.

AMPs are particularly attractive because resistance development appears less frequent compared with conventional antifungal agents.

Histatins

Histatins are salivary peptides with potent antifungal activity against *Candida* species.

Functions include:

- membrane permeabilization,
- mitochondrial dysfunction,
- inhibition of fungal adhesion,
- and biofilm suppression.

Defensins

Defensins are produced by epithelial cells and neutrophils and contribute to mucosal immune defense.

9.3 Nanoparticle-Based Drug Delivery Systems

Nanotechnology represents one of the most rapidly developing fields in antifungal therapy research. Nanoparticle-based systems may significantly improve antifungal drug delivery and therapeutic efficacy.

Advantages of Nanotechnology

Nanoparticles provide several advantages over conventional formulations:

- improved drug penetration,
- sustained release,
- enhanced stability,
- targeted delivery,
- reduced toxicity,
- and increased bioavailability.

Silver Nanoparticles

Silver nanoparticles possess strong antifungal and anti-biofilm activity.

Proposed mechanisms include:

- generation of reactive oxygen species,
- membrane disruption,

- DNA damage,
- and interference with fungal metabolism.

Chitosan Nanoparticles

Chitosan-based systems are particularly attractive in oral applications due to their:

- biocompatibility,
- mucoadhesive properties,
- biodegradability,
- and intrinsic antimicrobial activity.

Liposomal Drug Delivery

Liposomal formulations may reduce systemic toxicity while improving tissue penetration.

Liposomal amphotericin B represents one of the best-known clinical examples.

9.4 Photodynamic Therapy

Photodynamic therapy (PDT) has emerged as a promising minimally invasive treatment strategy for oral fungal infections.

PDT involves three key components:

1. Photosensitizer
2. Light source
3. Molecular oxygen

Activation of the photosensitizer by light generates reactive oxygen species that induce fungal cell death.

Advantages of PDT

- Minimal resistance development
- Localized therapeutic effect
- Effective biofilm disruption
- Reduced systemic toxicity
- Compatibility with combination therapy

Common Photosensitizers

- Methylene blue
- Toluidine blue
- Curcumin
- Rose bengal

PDT has demonstrated encouraging results in management of denture stomatitis and biofilm-associated oral candidiasis.

9.5 Probiotics and Oral Microbiome Modulation

The concept of microbiome modulation has gained increasing attention in oral fungal disease management.

Probiotics may suppress fungal colonization through:

- competitive adhesion,
- production of antimicrobial metabolites,
- pH modulation,
- and immune regulation.

Commonly investigated probiotic organisms include:

- *Lactobacillus rhamnosus*,
- *Lactobacillus reuteri*,
- *Bifidobacterium* species.

Several clinical studies have reported reductions in oral *Candida* carriage following probiotic administration.

9.6 Quorum Sensing Inhibitors

Quorum sensing pathways regulate fungal communication, virulence, morphogenesis, and biofilm maturation.

Targeting fungal signaling pathways represents an innovative strategy for reducing virulence without directly promoting selective pressure for resistance.

Farnesol

Farnesol inhibits hyphal transformation and biofilm development in *Candida albicans*.

9.7 Combination Therapy Approaches

Combination therapy strategies aim to:

- enhance antifungal efficacy,
- reduce drug resistance,
- lower drug toxicity,
- and improve biofilm penetration.

Examples include:

- azole + echinocandin,
- nystatin + thymoquinone,
- antifungal + nanoparticle,
- antifungal + photodynamic therapy.

Combination approaches are particularly promising in multidrug-resistant fungal infections.

10. Biofilm-Targeted Therapeutic Approaches

9.1 Natural Products and Phytotherapeutics

Naturally derived compounds have attracted considerable interest due to their antifungal, anti-inflammatory, antioxidant, and anti-biofilm properties.

Thymoquinone

Thymoquinone (TQ), the major bioactive component of *Nigella sativa*, has demonstrated significant antifungal activity against *Candida* species.

Reported mechanisms include:

- Cell membrane disruption
- Oxidative stress induction
- Biofilm inhibition
- Synergistic interaction with conventional antifungals

Recent studies have demonstrated synergistic antifungal activity between thymoquinone and nystatin against oral *Candida* isolates (Özdal Zincir et al., 2022).

Curcumin

Curcumin exhibits:

- Antifungal activity

- Anti-inflammatory effects
- Biofilm inhibition
- Photodynamic enhancement potential

Essential Oils

Examples:

- Tea tree oil
- Clove oil
- Cinnamon oil
- Oregano oil

9.2 Antimicrobial Peptides

Antimicrobial peptides (AMPs) represent promising alternatives to conventional antifungals.

Mechanisms:

- Membrane disruption
- Immunomodulation
- Biofilm inhibition

Examples:

- Histatins
- Defensins
- Cathelicidins

9.3 Nanoparticle-Based Therapies

Nanotechnology offers major advantages in antifungal drug delivery.

Advantages of Nanoparticles

- Enhanced drug penetration
- Sustained release
- Reduced toxicity
- Improved bioavailability
- Biofilm targeting

Types of Nanoparticles

- Silver nanoparticles
- Gold nanoparticles
- Chitosan nanoparticles
- Liposomal systems
- Polymeric nanoparticles

9.4 Photodynamic Therapy

Photodynamic therapy (PDT) combines:

- Photosensitizer
- Light source
- Oxygen

Reactive oxygen species generated during PDT induce fungal cell death.

Advantages:

- Minimal resistance development
- Localized action
- Biofilm disruption

9.5 Probiotics and Microbiome Modulation

Probiotics may restore microbial balance and inhibit fungal colonization.

Common probiotic species:

- *Lactobacillus rhamnosus*
- *Lactobacillus reuteri*
- *Bifidobacterium* species

Mechanisms:

- Competitive inhibition
- pH modulation
- Bacteriocin production
- Immune modulation

9.6 Quorum Sensing Inhibitors

Quorum sensing pathways regulate fungal virulence and biofilm formation.

Potential targets include:

- Farnesol signaling
- Tyrosol pathways
- Biofilm communication networks

9.7 Combination Therapy

Combination therapy may improve antifungal efficacy and reduce resistance.

Examples include:

- Azole + echinocandin
- Nystatin + thymoquinone
- Antifungal + PDT
- Antifungal + nanoparticles

10. Biofilm-Targeted Therapeutic Approaches

Biofilm-targeted therapy has become increasingly important because conventional antifungal agents are often insufficient against mature fungal biofilms.

Strategies include:

- Matrix degradation
- Enzyme inhibitors
- Surface modification
- Nanoparticle penetration systems
- Anti-adhesion coatings

11. Immunotherapy and Host-Directed Therapy

Host immune responses play central roles in fungal clearance.

Emerging immunotherapeutic approaches include:

- Cytokine therapy
- Monoclonal antibodies
- Vaccines
- Immune checkpoint modulation

12. Artificial Intelligence and Precision Medicine

Artificial intelligence (AI) and machine learning are increasingly used in:

- Antifungal susceptibility prediction

- Diagnostic imaging
- Microbiome analysis
- Personalized therapy planning

Precision medicine approaches may enable individualized antifungal treatment strategies.

13. Future Perspectives

Future antifungal strategies are expected to focus on:

- Personalized medicine
- Biofilm-targeted therapies
- Microbiome modulation
- Nanotechnology-based delivery systems
- Immunotherapy
- AI-supported diagnostics

The integration of multidisciplinary approaches will likely improve therapeutic outcomes and reduce resistance development.

14. Conclusion

Oral candidiasis remains a major clinical challenge due to increasing antifungal resistance, widespread biofilm-associated infections, and the growing prevalence of medically compromised patient populations. Although conventional antifungal therapies continue to play important roles in disease management, emerging resistance mechanisms substantially limit therapeutic success. Recent advances in molecular microbiology, nanotechnology, immunotherapy, microbiome research, and biomaterials have accelerated the development of innovative treatment approaches.

Natural compounds, antimicrobial peptides, nanoparticles, probiotics, photodynamic therapy, quorum sensing inhibitors, and synergistic combination regimens represent promising alternatives or adjuncts to conventional antifungal therapies. In particular, biofilm-targeted strategies and personalized medicine approaches are expected to become increasingly important in future clinical practice.

A comprehensive understanding of fungal virulence, host immunity, microbial ecology, and resistance mechanisms is essential for the development of more effective therapeutic strategies. Continued interdisciplinary research will play a critical role in overcoming antifungal resistance and improving clinical outcomes in oral candidiasis.

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Guided Bone Regeneration in Oral Surgery: Biomaterials, Biological Principles, and Clinical Outcomes

Özge Özdal Zincir¹

1. Introduction

Guided bone regeneration (GBR) represents one of the most significant advances in regenerative oral and maxillofacial surgery and has become a cornerstone of contemporary implant and reconstructive treatment protocols (Buser et al., 1993; Dahlin et al., 1989; Urban et al., 2019). The increasing demand for implant-supported rehabilitation, esthetic reconstruction, and functional restoration has considerably expanded the indications for alveolar bone augmentation procedures (Buser, Dula, Belser, Hirt, & Berthold, 1993; Dahlin, Sennerby, Lekholm, Linde, & Nyman, 1989).

The biological concept underlying GBR is derived from the principle of selective cell exclusion. Barrier membranes are used to prevent rapid migration of epithelial and connective tissue cells into osseous defects while allowing slower-growing osteogenic cells to repopulate the regenerative space (Nyman, Lindhe, Karring, & Rylander, 1982). Maintenance of a secluded environment favorable for osteogenesis is therefore fundamental for successful bone regeneration.

Over the past several decades, major developments in biomaterials science, tissue engineering, growth factor biology, and surgical techniques have transformed GBR into a highly predictable regenerative procedure. Contemporary GBR protocols involve sophisticated interactions among barrier membranes, bone graft substitutes, osteogenic cells, angiogenesis, inflammatory mediators, and molecular signaling pathways.

Bone deficiency within the craniofacial skeleton may arise from trauma, tooth loss, periodontal disease, infection, congenital deformities, cystic lesions, tumor resection, or physiological alveolar resorption following extraction (Araujo & Lindhe, 2005; Tan et al., 2012). Inadequate bone volume may compromise implant placement, esthetic outcomes, and long-term functional stability. Consequently, regenerative procedures aimed at restoring alveolar bone architecture have become increasingly important.

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Autogenous bone grafts have historically been regarded as the gold standard because of their osteogenic, osteoinductive, and osteoconductive properties (Aghaloo & Moy, 2007; Triplett & Schow, 1996). However, limitations including donor site morbidity, limited graft availability, unpredictable resorption, and increased surgical complexity have stimulated development of alternative biomaterials including allografts, xenografts, alloplastic substitutes, and tissue-engineered scaffolds (Aghaloo & Moy, 2007).

In addition to graft materials, barrier membranes play central roles in space maintenance and tissue compartmentalization. Membrane technologies have evolved considerably from early non-resorbable expanded polytetrafluoroethylene (ePTFE) barriers to advanced collagen-based and bioactive membranes with improved biocompatibility and regenerative potential.

Recent advances in regenerative medicine have further expanded the field of GBR. Novel approaches involving platelet concentrates, stem cell therapy, recombinant growth factors, gene therapy, nanotechnology, three-dimensional printing, and customized scaffold fabrication have opened new perspectives for oral bone regeneration.

The purpose of this chapter is to provide a comprehensive and evidence-based review of guided bone regeneration in oral surgery. Biological principles, wound healing dynamics, graft biomaterials, membrane technologies, clinical applications, complications, and long-term treatment outcomes are critically discussed.

1.1 Historical Development of Guided Bone Regeneration

The concept of guided tissue regeneration was initially developed in periodontology following observations that selective cell repopulation could influence periodontal wound healing outcomes. Early studies by Nyman and colleagues demonstrated that exclusion of epithelial cells facilitated periodontal ligament regeneration rather than repair by long junctional epithelium.

These principles were subsequently adapted for osseous regeneration in implant dentistry and oral surgery. Dahlin et al. (1988) demonstrated that barrier membranes could successfully promote bone regeneration around implants and osseous defects. This work established the biological basis of modern GBR.

Since then, regenerative protocols have evolved substantially. Early reliance on autogenous grafts and non-resorbable membranes has gradually expanded toward use of xenografts, synthetic biomaterials, growth factors, platelet concentrates, and bioengineered scaffolds.

1.2 Clinical Significance in Oral Surgery

GBR is now routinely used in multiple clinical scenarios including:

- implant site development,
- horizontal ridge augmentation,

- vertical ridge augmentation,
- ridge preservation,
- peri-implant defect management,
- sinus floor augmentation,
- periodontal regeneration,
- cystic defect reconstruction,
- and reconstruction following trauma or tumor resection.

The ability to regenerate deficient alveolar bone has significantly improved implant treatment possibilities in anatomically compromised patients.

1.3 Scope of the Chapter

This chapter focuses on:

- biological principles of GBR,
- wound healing mechanisms,
- angiogenesis and osteogenesis,
- biomaterials and graft substitutes,
- membrane technologies,
- growth factors and biologics,
- tissue engineering approaches,
- clinical indications,
- complications and risk factors,
- and long-term clinical outcomes.

Special emphasis is placed on translational regenerative strategies and emerging technologies likely to shape the future of oral bone regeneration.

2. Biological Principles of Guided Bone Regeneration

The biological foundation of guided bone regeneration is based on principles of wound healing, cellular competition, tissue compartmentalization, angiogenesis, and osteogenesis. Successful regeneration requires highly coordinated interactions among inflammatory mediators, progenitor cells, extracellular matrix proteins, vascular structures, mechanical stability, and biomaterial properties.

Bone healing within the craniofacial region differs substantially from regeneration in other skeletal sites because oral tissues are continuously exposed to bacterial biofilms, salivary contamination, functional loading, and dynamic soft tissue forces. Consequently, regenerative strategies in oral surgery must simultaneously address both biological and mechanical challenges.

The regenerative cascade begins immediately following surgical injury and involves sequential inflammatory, proliferative, angiogenic, osteogenic, and remodeling phases. Platelet activation and clot formation provide an initial fibrin scaffold that stabilizes the wound and facilitates recruitment of inflammatory cells and mesenchymal stem cells. Cytokines and growth factors released during the inflammatory phase regulate cellular migration, differentiation, and angiogenesis.

Several molecular mediators play critical roles in GBR-associated regeneration:

- transforming growth factor beta (TGF- β),
- vascular endothelial growth factor (VEGF),
- platelet-derived growth factor (PDGF),
- bone morphogenetic proteins (BMPs),
- fibroblast growth factor (FGF),
- and insulin-like growth factors (IGFs).

Macrophages also play central roles in regenerative healing. Early inflammatory M1 macrophages contribute to debridement and antimicrobial defense, whereas later M2 macrophages support tissue regeneration, angiogenesis, and extracellular matrix remodeling. Contemporary regenerative research increasingly focuses on immunomodulatory approaches aimed at optimizing macrophage polarization and inflammatory regulation.

2.1 Bone Biology and Remodeling

2.1 Bone Biology and Remodeling

Bone is a highly dynamic tissue undergoing continuous remodeling through coordinated activity of osteoblasts, osteoclasts, osteocytes, inflammatory cells, vascular structures, and extracellular matrix proteins.

The regenerative capacity of bone depends on:

- cellular viability,
- vascular supply,
- mechanical stability,
- signaling molecules,
- and host immune responses.

Bone healing involves overlapping inflammatory, reparative, and remodeling phases.

2.2 Principles of Selective Cell Exclusion

Selective exclusion of rapidly proliferating epithelial and fibroblastic cells is fundamental to GBR.

Barrier membranes create a secluded environment allowing migration of:

- osteogenic cells,
- mesenchymal stem cells,
- and vascular elements.

2.3 Space Maintenance

Successful regeneration requires maintenance of a stable regenerative space.

Space collapse may result in:

- fibrous tissue infiltration,
- incomplete bone formation,
- and graft failure.

2.4 Angiogenesis and Vascularization

Adequate vascularization is considered one of the most critical determinants of successful bone regeneration because oxygen supply, nutrient delivery, inflammatory regulation, stem cell migration, and mineralization are all dependent on microvascular development (Sculean et al., 2019; Wang & Boyapati, 2006). Following graft placement, angiogenic processes begin rapidly and are mediated through complex signaling pathways involving vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), and hypoxia-inducible factors.

Insufficient vascularization may result in:

- graft necrosis,
- delayed remodeling,
- incomplete mineralization,
- infection,
- and fibrous tissue encapsulation.

Particulate grafts generally demonstrate superior revascularization compared with dense cortical blocks because increased surface area facilitates cellular infiltration and capillary ingrowth (Jensen & Terheyden, 2009).

Recent studies have highlighted the importance of angiogenic modulation in regenerative medicine. Biomaterials capable of controlled release of VEGF and other angiogenic mediators may significantly improve regenerative outcomes in large or poorly vascularized defects.

2.5 Immunological Aspects of Bone Regeneration

The immune system plays a central role in tissue regeneration and biomaterial integration. Contemporary regenerative concepts increasingly emphasize the

field of osteoimmunology, which investigates interactions between immune responses and bone metabolism.

Macrophages are among the most important regulators of regenerative healing. Early inflammatory M1 macrophages participate in microbial defense and tissue debridement, whereas M2 macrophages promote angiogenesis, extracellular matrix synthesis, and osteogenic differentiation.

Excessive or prolonged inflammation may negatively affect regeneration through:

- osteoclastic activation,
- fibrous tissue formation,
- cytokine dysregulation,
- and impaired angiogenesis.

Consequently, modulation of inflammatory pathways has become an important target in advanced GBR research.

2.6 Mechanical Stability and Regeneration

Adequate vascularization is essential for oxygen delivery, nutrient transport, inflammatory regulation, and osteogenesis.

Growth factors involved include:

- VEGF,
- PDGF,
- TGF- β ,
- BMPs.

3. Bone Graft Materials

Bone graft biomaterials constitute essential components of GBR procedures and are used to support regeneration through osteogenesis, osteoinduction, osteoconduction, or combinations of these biological mechanisms. The ideal graft material should possess biocompatibility, structural stability, space-maintaining capacity, predictable remodeling characteristics, and minimal immunogenicity.

Selection of graft materials depends on multiple factors including:

- defect morphology,
- defect size,
- vascularity,
- implant timing,
- soft tissue quality,
- patient systemic status,

- and surgeon preference.

Contemporary regenerative surgery increasingly favors combination grafting strategies in which different biomaterials are mixed to optimize biological and mechanical properties. For example, autogenous bone particles may be combined with xenografts to reduce resorption while maintaining osteogenic potential.

3.1 Autogenous Bone Grafts

Autogenous grafts remain the reference standard in bone regeneration because they contain viable osteogenic cells, endogenous growth factors, and native bone matrix proteins capable of promoting new bone formation (Misch, 2010; Triplett & Schow, 1996).

Autogenous grafts may be harvested as:

- particulate grafts,
- cortical blocks,
- cancellous grafts,
- corticocancellous grafts,
- or bone chips.

Biological Properties

Autogenous grafts possess:

- osteogenesis through viable osteoblasts and progenitor cells,
- osteoinduction through endogenous signaling molecules,
- and osteoconduction through natural bone scaffold architecture.

Intraoral Donor Sites

Common intraoral donor sites include:

- mandibular ramus,
- symphysis,
- maxillary tuberosity,
- zygomatic buttress.

Intraoral harvesting offers advantages such as reduced morbidity, shorter operative time, and embryologic similarity to recipient sites.

Extraoral Donor Sites

Extraoral donor sites may be necessary in severe atrophy cases.

These include:

- iliac crest,
- calvarium,

- tibia,
- fibula.

However, extraoral harvesting is associated with increased morbidity and hospitalization.

Graft Resorption

One of the major limitations of autogenous grafts is unpredictable resorption. Cortical grafts generally exhibit slower resorption but reduced revascularization compared with cancellous grafts.

Several factors influence graft stability:

- fixation rigidity,
- vascularization,
- mechanical loading,
- smoking,
- infection,
- and soft tissue management.

3.2 Allografts

3.1 Autogenous Bone Grafts

Autogenous bone remains the gold standard in regenerative surgery due to its:

- osteogenic,
- osteoinductive,
- and osteoconductive properties.

Donor Sites

Intraoral donor sites:

- mandibular ramus,
- symphysis,
- maxillary tuberosity.

Extraoral donor sites:

- iliac crest,
- calvarium,
- tibia.

Advantages

- High biological activity

- Excellent regenerative potential
- No immunogenicity

Limitations

- Donor site morbidity
- Increased surgical time
- Limited availability
- Unpredictable resorption

3.2 Allografts

Allografts are derived from human donors and processed to reduce immunogenicity.

Types include:

- FDBA,
- DFDBA.

3.3 Xenografts

Xenografts are commonly bovine-derived materials with slow resorption characteristics.

3.4 Alloplastic Materials

Synthetic graft substitutes include:

- hydroxyapatite,
- β -tricalcium phosphate,
- bioactive glass,
- calcium sulfate.

Table 1. Characteristics of Bone Graft Materials

Material	Osteogenic	Osteoinductive	Osteoconductive
Autograft	Yes	Yes	Yes
Allograft	No	Variable	Yes
Xenograft	No	No	Yes
Alloplast	No	No	Yes

4. Barrier Membranes

Barrier membranes are fundamental components of guided bone regeneration because they create a protected regenerative environment and prevent soft tissue invasion into osseous defects. Membranes function by

selectively excluding epithelial and fibroblastic cells while allowing slower-growing osteogenic cells to repopulate the defect area.

An ideal membrane should possess:

- biocompatibility,
- tissue integration,
- space maintenance capability,
- selective permeability,
- mechanical stability,
- ease of handling,
- and controlled degradation characteristics.

Membrane performance is strongly influenced by defect morphology, graft material selection, flap design, vascular supply, and surgical stability.

4.1 Non-Resorbable Membranes

4.1 Non-Resorbable Membranes

ePTFE Membranes

Expanded polytetrafluoroethylene membranes were among the first GBR barriers.

Advantages:

- Excellent space maintenance
- Mechanical stability

Limitations:

- Need for second surgery
- High complication risk if exposed

Titanium-Reinforced Membranes

Titanium reinforcement improves vertical augmentation stability.

4.2 Resorbable Membranes

Collagen membranes are currently the most widely used barriers in contemporary GBR procedures because of their favorable biocompatibility and clinical handling characteristics (Rothamel et al., 2005; Tal et al., 2008).

Advantages include:

- Biocompatibility
- Reduced morbidity
- No second surgery

Limitations include:

- Reduced rigidity
- Faster degradation

4.3 Emerging Membrane Technologies

Novel membrane strategies include:

- multilayer membranes,
- bioactive membranes,
- antimicrobial coatings,
- nanofiber membranes,
- growth factor-loaded membranes.

5. Biological Mediators and Growth Factors

Biological mediators and growth factors play essential roles in regulating cellular migration, differentiation, extracellular matrix synthesis, angiogenesis, and osteogenesis during bone regeneration. Increasing understanding of molecular signaling pathways has substantially improved regenerative strategies in oral surgery.

Growth factors may be delivered through:

- recombinant proteins,
- platelet concentrates,
- biomaterial carriers,
- stem cell scaffolds,
- or gene therapy systems.

5.1 Bone Morphogenetic Proteins

5.1 Bone Morphogenetic Proteins

BMPs are potent osteoinductive molecules.

BMP-2 is widely investigated in oral regeneration.

5.2 Platelet-Derived Growth Factor

PDGF stimulates:

- angiogenesis,
- fibroblast proliferation,
- osteoblastic activity.

5.3 Platelet Concentrates

Platelet concentrates have become increasingly popular adjuncts in regenerative oral surgery because they provide autologous sources of growth factors, cytokines, fibrin matrices, and leukocytes capable of enhancing wound healing and angiogenesis (Choukroun et al., 2006; Simonpieri et al., 2012).

The most commonly used platelet concentrates include:

- platelet-rich plasma (PRP),
- platelet-rich fibrin (PRF),
- advanced platelet-rich fibrin (A-PRF),
- and injectable platelet-rich fibrin (i-PRF).

Biological Effects

Platelet concentrates may contribute to:

- angiogenesis,
- fibroblast proliferation,
- collagen synthesis,
- soft tissue healing,
- and early mineralization.

Growth factors released include:

- PDGF,
- TGF- β ,
- VEGF,
- IGF,
- and EGF.

Platelet-Rich Fibrin

PRF has become particularly popular in oral surgery because of its simplified preparation and favorable handling characteristics.

Advantages include:

- autologous origin,
- low cost,
- ease of preparation,
- and prolonged release of growth factors.

Clinical applications include:

- sinus augmentation,

- ridge preservation,
- periodontal regeneration,
- peri-implant defect treatment,
- and soft tissue enhancement.

However, despite promising biological properties, the literature remains heterogeneous regarding long-term superiority of platelet concentrates over conventional GBR procedures.

5.4 Recombinant Growth Factors

PRP and PRF

Platelet concentrates release:

- growth factors,
- cytokines,
- and fibrin scaffolds.

Potential benefits include:

- accelerated healing,
- enhanced angiogenesis,
- reduced inflammation.

6. Tissue Engineering and Stem Cell Approaches

Tissue engineering has emerged as one of the most promising fields in regenerative medicine and aims to restore biological function through integration of cells, scaffolds, and signaling molecules. In oral and maxillofacial surgery, tissue engineering approaches are increasingly investigated as alternatives to conventional grafting procedures.

The classic tissue engineering triad includes:

1. Cells
2. Scaffolds
3. Signaling molecules

Successful regeneration requires coordination among these components within a mechanically stable and vascularized environment.

6.1 Mesenchymal Stem Cells

Tissue engineering integrates:

- scaffolds,
- cells,
- signaling molecules.

6.1 Mesenchymal Stem Cells

MSCs may differentiate into:

- osteoblasts,
- chondrocytes,
- adipocytes.

Sources include:

- bone marrow,
- adipose tissue,
- dental pulp,
- periodontal ligament.

6.2 Three-Dimensional Printing

Three-dimensional printing technologies have rapidly expanded in regenerative surgery and now allow fabrication of customized patient-specific scaffolds, surgical guides, titanium meshes, and biomaterial constructs.

Additive manufacturing techniques offer several advantages:

- precise anatomical adaptation,
- improved surgical planning,
- reduced operative time,
- enhanced scaffold architecture,
- and individualized regenerative design.

Customized titanium meshes generated using digital workflows may improve vertical ridge augmentation outcomes by enhancing space maintenance and reducing intraoperative adjustment requirements.

Bioprinting

Bioprinting technologies aim to integrate:

- living cells,
- hydrogels,
- biomaterials,
- and signaling molecules.

Although still largely experimental in oral surgery, bioprinting may eventually permit fabrication of biologically active personalized bone constructs.

Limitations

Current limitations include:

- high production costs,
- regulatory challenges,
- limited long-term evidence,
- and difficulties in achieving predictable vascularization.

6.3 Gene Therapy and Molecular Engineering

3D printing technologies enable:

- customized scaffold fabrication,
- patient-specific graft design,
- precise anatomical adaptation.

7. Clinical Applications of GBR

Guided bone regeneration has become widely integrated into routine oral and implant surgical practice. Clinical indications continue to expand as regenerative techniques and biomaterials evolve.

The predictability of GBR depends on:

- defect configuration,
- blood supply,
- soft tissue management,
- biomaterial stability,
- membrane adaptation,
- and patient compliance.

Careful surgical planning and flap management remain essential for long-term regenerative success.

7.1 Ridge Preservation

7.1 Ridge Preservation

Alveolar ridge resorption following extraction may compromise implant placement.

Ridge preservation techniques aim to minimize dimensional changes.

7.2 Horizontal Ridge Augmentation

Horizontal deficiency is among the most common indications for GBR.

7.3 Vertical Ridge Augmentation

Vertical augmentation remains technically demanding due to:

- gravitational forces,
- soft tissue tension,

- vascular limitations.

7.4 Sinus Floor Augmentation

Maxillary sinus augmentation represents one of the most predictable regenerative procedures in implant dentistry and frequently incorporates GBR principles.

Posterior maxillary bone deficiency commonly results from:

- sinus pneumatization,
- periodontal disease,
- long-term edentulism,
- and physiological ridge resorption.

Sinus augmentation procedures may be performed using:

- lateral window approaches,
- transcrestal techniques,
- simultaneous implant placement,
- or staged protocols.

A wide variety of graft materials have been investigated in sinus augmentation including autogenous grafts, xenografts, allografts, alloplasts, and combinations thereof.

Histological Outcomes in Sinus Augmentation

Histological studies demonstrate substantial variation in new bone formation according to biomaterial selection and healing duration. Xenografts frequently demonstrate persistence of residual particles even after prolonged healing periods, whereas autogenous grafts exhibit more rapid remodeling.

Complications

Potential complications include:

- sinus membrane perforation,
- infection,
- graft migration,
- sinusitis,
- and implant instability.

7.5 Peri-Implant Regeneration

Maxillary sinus augmentation is frequently combined with GBR principles.

7.5 Peri-Implant Defect Regeneration

GBR may be used in treatment of peri-implantitis-related osseous defects.

8. Complications and Risk Factors

Although GBR is generally considered predictable, complications remain relatively common and may significantly compromise regenerative outcomes. Complication rates vary depending on defect morphology, augmentation size, membrane type, graft material, flap design, surgical experience, and patient-related risk factors.

The most commonly reported complications include:

- membrane exposure,
- infection,
- graft contamination,
- soft tissue dehiscence,
- graft resorption,
- insufficient bone formation,
- and implant failure.

Smoking, poorly controlled diabetes, inadequate plaque control, and thin gingival biotype may substantially increase complication risk.

8.1 Membrane Exposure

8.1 Membrane Exposure

Membrane exposure remains one of the most common GBR complications.

Consequences include:

- contamination,
- infection,
- graft loss,
- incomplete regeneration.

8.2 Infection

Postoperative infection may compromise regenerative outcomes.

8.3 Graft Resorption

Excessive graft resorption may reduce final bone volume.

8.4 Soft Tissue Dehiscence

Primary closure is essential for successful GBR.

9. Clinical Outcomes and Evidence-Based Perspectives

Long-term clinical evidence generally supports the effectiveness of guided bone regeneration in oral surgery and implant rehabilitation. Numerous systematic reviews and longitudinal studies have demonstrated favorable

outcomes regarding horizontal augmentation, ridge preservation, peri-implant regeneration, and sinus floor augmentation procedures.

However, regenerative outcomes remain highly variable among patients and clinical scenarios. Differences in biomaterial selection, membrane design, defect morphology, surgical technique, follow-up duration, and patient systemic health substantially influence treatment success.

9.1 Horizontal Ridge Augmentation Outcomes

Horizontal ridge augmentation demonstrates among the highest predictability rates in GBR.

Reported benefits include:

- increased ridge width,
- improved implant positioning,
- enhanced prosthetic outcomes,
- and improved esthetic stability.

9.2 Vertical Ridge Augmentation Outcomes

Vertical augmentation remains more challenging than horizontal augmentation because of:

- limited vascularity,
- soft tissue tension,
- gravitational forces,
- and difficulty maintaining regenerative space.

Nevertheless, contemporary studies have reported encouraging outcomes using titanium-reinforced membranes and staged regenerative protocols.

9.3 Histological Outcomes

Histological analyses demonstrate that regenerated bone quality varies according to biomaterial selection and healing duration.

Autogenous grafts generally exhibit:

- faster remodeling,
- higher cellularity,
- and more mature lamellar bone formation.

Xenografts typically demonstrate slower remodeling and persistence of residual graft particles over extended periods.

9.4 Implant Survival Following GBR

Implant survival rates following GBR procedures are generally high and frequently exceed 90% in long-term studies when proper surgical protocols are followed.

10. Emerging Technologies and Future Directions

Long-term studies have generally demonstrated favorable outcomes for GBR procedures when appropriate case selection and surgical principles are followed (Buser et al., 2002; Simion et al., 2007; Urban et al., 2019).

Factors influencing success include:

- defect morphology,
- biomaterial selection,
- surgical technique,
- patient compliance,
- smoking,
- systemic health.

Systematic reviews have demonstrated high implant survival rates following GBR-assisted augmentation procedures.

10. Emerging Technologies and Future Directions

Future regenerative strategies are expected to focus on:

- bioactive scaffolds,
- smart biomaterials,
- controlled growth factor delivery,
- nanotechnology,
- stem cell therapy,
- gene therapy,
- artificial intelligence-assisted planning,
- personalized regenerative medicine.

Immunomodulatory approaches targeting macrophage polarization and inflammatory regulation may further improve regenerative outcomes.

11. Conclusion

Guided bone regeneration has become an indispensable component of contemporary oral and maxillofacial surgery. Advances in biomaterials, membrane technologies, growth factors, tissue engineering, and regenerative biology have substantially improved clinical predictability and treatment outcomes. Nevertheless, successful regeneration remains highly dependent on

appropriate case selection, meticulous surgical technique, defect morphology, vascularization, biomaterial choice, and patient-related factors.

Although autogenous bone grafting continues to represent the biological gold standard, alternative biomaterials and bioengineered regenerative approaches have significantly expanded clinical possibilities while reducing morbidity. Emerging strategies involving stem cells, nanotechnology, bioactive membranes, and personalized regenerative medicine are likely to further transform the field of oral bone regeneration.

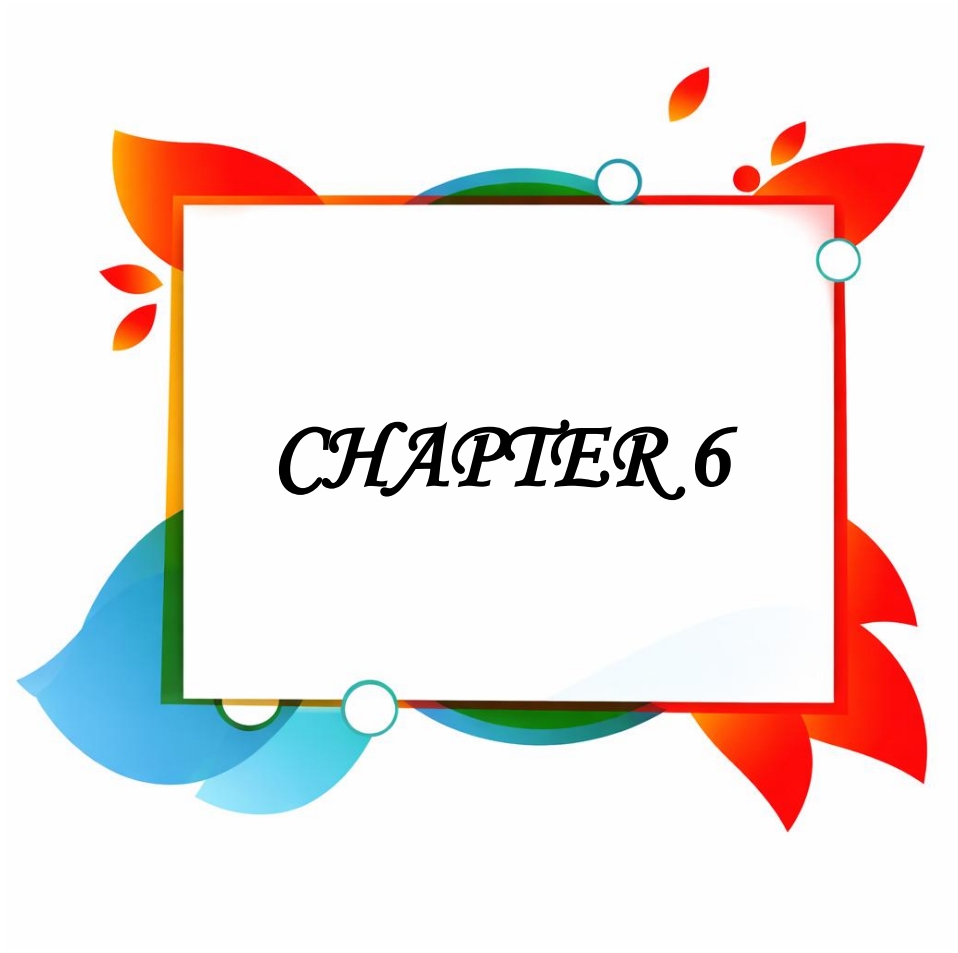
Continued interdisciplinary research integrating biology, engineering, biomaterials science, and clinical surgery will remain essential for optimizing regenerative outcomes and developing next-generation GBR technologies.

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Biophysical Effects of TENS and NMES Applications

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1. Introduction

Transcutaneous Electrical Nerve Stimulation (TENS) and Neuromuscular Electrical Stimulation (NMES) are two widely used electrotherapy modalities in contemporary clinical practice. Both techniques are based on the principle of delivering electrical current to biological tissues via surface electrodes placed on the skin (Duymaz, 2018; Karaahmetoğlu and Pehlivan, 2024). While TENS is primarily used for pain control, NMES is preferred to enhance muscle activation, support muscle strength, and improve functional mobility (Duymaz, 2018; Özer, 2023).

The effects of electrical current on neural and muscular tissues are explained through biophysical mechanisms. Key stimulation parameters such as current intensity, pulse duration, and pulse frequency directly influence nerve fiber excitability thresholds, action potential generation, and the electrical responsiveness of muscle tissue (Duymaz, 2018; Özer, 2023). In TENS applications, high-frequency stimulation (>50 Hz) is reported to produce analgesic effects through the gate control mechanism, whereas low-frequency stimulation (≤ 10 Hz) contributes to analgesia by enhancing endogenous opioid release (Özen and Koç, 2023; Özer, 2023). In NMES, the synchronous and non-selective activation of motor units leads to a pattern of muscle fiber recruitment that differs from voluntary contraction, thereby exerting a direct effect on muscle strength and functional performance (Bickel et al., 2011; Duymaz, 2018; Kazak, 2023).

In recent years, there has been a marked increase in clinical and experimental studies investigating TENS and NMES applications (Çakır et al., 2024; Karaahmetoğlu and Pehlivan, 2024). However, current evidence suggests that clinical effectiveness may vary depending on patient population, stimulation dosage, and study design (Vance et al., 2022). This highlights the importance of

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evaluating electrical stimulation techniques not only in terms of clinical outcomes but also through their underlying biophysical mechanisms.

The aim of this chapter is to explain the biophysical foundations of TENS and NMES applications and evaluate the effects of electrical stimulation parameters on neural and muscular tissues in light of the current literature.

2. Biophysical Foundations

2.1. Effects of Electrical Stimulation on Tissues

In electrical stimulation applications, neural and muscular tissues respond to electrical currents due to their excitable membrane properties. Electrical stimulation induces changes in membrane potential by activating peripheral nerves and muscle fibers. These alterations at the cellular level may subsequently influence protein synthesis, enzymatic activity, and various structural responses within the tissue. Such biophysical processes constitute the fundamental basis of the therapeutic effects observed with electrical stimulation (Kazak, 2023).

2.2. Levels of Action of Electrical Stimulation

The effects of electrical stimulation can be evaluated at cellular, tissue, segmental, and systemic levels. At the cellular level, the primary effect begins with changes in membrane potential in nerve and muscle cells. At the tissue level, electrical stimulation is reported to enhance muscle contraction, thereby contributing to improvements in muscle strength, accelerating venous and lymphatic circulation, and supporting tissue regeneration. At the segmental level, effects occur through group muscle contractions and synergistic muscle activation patterns. This mechanism supports joint mobility and indirectly enhances blood flow and lymphatic drainage through the muscle pump effect. At the systemic level, electrical stimulation is suggested to modulate the release of endogenous substances and various neurotransmitters associated with analgesic pathways, thereby contributing to a reduction in pain perception (Özen and Koç, 2023; Özer, 2023; Kazak, 2023).

2.3. Fundamental Mechanisms of Electrical Stimulation

The physiological effects of electrical stimulation are explained through electrophysical, electrochemical, and electrothermal mechanisms. Electrophysical effects are associated with the generation and propagation of action potentials in nerve and muscle cell membranes. Electrochemical effects arise at the cellular level through ion movements, changes in membrane permeability, and various biochemical processes. Electrothermal effects occur as a result of heat generation within tissues induced by electrical current. The combined interaction of these mechanisms underlies the biological and clinical effects of electrical stimulation (Kazak, 2023).

2.4. Electrical Stimulation Parameters

The biophysical response to electrical stimulation depends on both the stimulation parameters and the type of targeted tissue. Frequency, amplitude, pulse duration, waveform, duty cycle (on–off time), and electrode placement are among the primary parameters determining the physiological response (Doucet et al., 2012; Kazak, 2023).

In particular, in NMES applications, these parameters have been reported to directly influence force production, motor unit recruitment patterns, and the development of muscle fatigue (Doucet et al., 2012). Therefore, appropriate adjustment of stimulation parameters is considered essential for enhancing therapeutic effectiveness (Duymaz, 2018; Özer, 2023). Furthermore, it has been stated that the accuracy of reported parameter values in electrical stimulation devices, as well as the methodological characteristics of these devices, has been relatively underexplored in the literature (Guimarães et al., 2021).

2.4.1. Frequency

Frequency refers to the number of electrical impulses (pulses) delivered per unit time and is typically expressed in Hertz (Hz). In conventional TENS applications, high frequencies in the range of 80–100 Hz are commonly used, whereas low-frequency, acupuncture-like TENS is typically applied at approximately 2 Hz. High-frequency TENS is generally intended to produce analgesia at the sensory level without inducing muscle contractions. In contrast, low-frequency applications, when delivered at higher intensities, may produce visible muscle twitches (Howarth, 2011; Özer, 2023).

In NMES applications, frequencies between 20–50 Hz are commonly preferred to elicit effective muscle contractions (Doucet et al., 2012). Within this range, successive stimuli can produce temporal summation in muscle fibers, leading to tetanic contractions. At lower frequencies, each electrical impulse induces an individual contraction–relaxation response, resulting in twitch-type contractions. Although these twitch contractions generate lower force compared to tetanic contractions, they may still induce significant metabolic changes in muscle tissue (Rege et al., 2026). High-frequency NMES has been associated with increased metabolic demand and a more rapid onset of muscle fatigue. This phenomenon has been suggested to be related to the continuous activation of motor units without sufficient rotation among muscle fibers (Bickel et al., 2011).

2.4.2. Amplitude (Current Intensity)

Amplitude refers to the intensity of the applied electrical current and is typically measured in milliamperes (mA). The literature indicates a generally positive relationship between amplitude and muscle contraction force. Therefore, current intensity is gradually increased until the desired level of muscle contraction is achieved. In NMES applications, since the primary goal is to elicit

muscle contraction, the current is increased above the motor threshold until a visible contraction is obtained (Duymaz, 2018; Kazak, 2023). Higher amplitude values may recruit a greater number of motor units, thereby contributing to increased force production. However, excessively high intensities may reduce patient comfort and accelerate neuromuscular fatigue (Doucet et al., 2012).

In TENS applications, current intensity is typically adjusted according to the individual's sensory threshold. The optimal stimulation level for analgesic effect is generally described as a “strong but comfortable” sensory intensity. Clinical studies suggest that the most ecologically valid time frame for evaluating TENS effectiveness is during stimulation or immediately afterward (“in-the-moment”) (Johnson et al., 2022). In conventional TENS, stimulation is typically applied at sensory levels without inducing muscle contraction. However, in low-frequency, acupuncture-like TENS, higher intensities may result in mild muscle twitches (Howarth, 2011).

2.4.3. Pulse Duration / Phase Duration

Pulse duration refers to the time during which a single electrical pulse is delivered, and it is typically expressed in milliseconds (ms). In the literature, pulse duration values are reported to range between 1–300 ms, with this range being commonly used particularly for the activation of fully innervated muscles (Kazak, 2023). The selection of pulse duration is critical, as it influences the amount of charge delivered per pulse and, consequently, the excitability of neural and muscular tissues.

2.4.4. Waveform (Monophasic/Biphasic) and Modulation

Waveforms used in electrical stimulation can be monophasic or biphasic in nature. Alongside waveform characteristics, modulation type is also an important parameter that influences the biophysical response of tissues to electrical stimulation (Kazak, 2023). In clinical practice, biphasic waveforms are more commonly preferred due to their more balanced current distribution and improved patient comfort, as they reduce the risk of net charge accumulation in tissues (Duymaz, 2018).

2.4.5. On–Off and Ramp Times (Rise–Fall Times)

On–off times and ramp (rise–fall) times are important parameters that influence muscle performance and fatigue development during electrical stimulation. Appropriate adjustment of contraction and relaxation intervals is particularly important for minimizing muscle fatigue. Clinically, it is recommended that longer rest periods be used at the beginning of treatment, with a gradual increase in stimulation (on) time as therapy progresses (Duymaz, 2018; Kazak, 2023).

Ramp time refers to the duration required for the current to increase from zero to its maximum intensity and subsequently return to zero. Shorter rise times are generally preferred in order to achieve more effective and comfortable muscle contractions, as abrupt transitions may enhance the efficiency of motor unit recruitment while maintaining tolerability (Duymaz, 2018; Kazak, 2023).

2.4.6. Electrode Placement and Size

In TENS applications, electrodes are typically placed over the painful area, along dermatomal distributions, over the course of peripheral nerves, or at acupuncture points (Howarth, 2011; Vance et al., 2022; Özer, 2023). In interventions aiming for a segmental effect, local placement over the painful region is generally preferred, although contralateral or wider-area placements may also be used depending on the clinical objective (Johnson, 2007).

In NMES applications, placement of electrodes over the motor point is reported to produce more effective muscle contractions and higher force output (Maffiuletti et al., 2013; Doucet et al., 2012). This approach allows sufficient muscle contraction to be achieved with lower current intensities (Kazak, 2023; Duymaz, 2018).

Electrode size is another important factor influencing tissue response. Electrode size determines current density and thereby affects the spatial distribution of stimulation within the tissue. Smaller electrodes generate higher current density, whereas larger electrodes distribute the current over a broader area (Doucet et al., 2012). Additionally, variations in inter-electrode distance can influence the distribution of activated muscle fibers and the resulting force production (Doucet et al., 2012; Maffiuletti et al., 2013).

2.5. Biophysical Basis of TENS Applications

Several theories have been proposed in the literature to explain the analgesic effects of TENS, which aims to activate sensory nerve fibers to achieve pain relief. Among these, the most widely accepted is the Gate Control Theory. According to this theory, stimulation of large-diameter, fast-conducting A-beta fibers induces presynaptic inhibition at the dorsal horn of the spinal cord, thereby suppressing pain transmission from small-diameter nociceptive C fibers. As a result, the transmission of pain signals to higher centers is reduced, leading to an analgesic effect (Howarth, 2011; Özen and Koç, 2023; Özer, 2023).

In conventional TENS applications, analgesia typically occurs at sensory threshold levels without inducing muscle contraction. The effect has a rapid onset but is generally short-lived, and is primarily mediated at the segmental level via the gate control mechanism (Johnson, 2007; Howarth, 2011). However, the analgesic effects of TENS cannot be explained by a single mechanism alone. Depending on stimulation parameters, descending inhibitory pain control systems at supraspinal levels may also be activated. In particular, low-frequency, higher-intensity TENS has been reported to stimulate small-diameter afferent fibers, increase endogenous opioid release, and produce analgesia through descending modulatory pathways (Johnson, 2007; Howarth, 2011; Özen and Koç, 2023; Özer, 2023).

In addition, TENS may also exert peripheral effects, including local vasodilation, which may contribute to pain modulation. In some application modes, stimulation of acupuncture points has also been suggested to enhance analgesic effects (Özen and Koç, 2023; Özer, 2023; Kazak, 2023).

Recent electrophysiological studies have demonstrated that TENS can also induce measurable cortical changes. In particular, low-frequency TENS has been shown to increase alpha-band (8–12 Hz) oscillations in the primary somatosensory cortex (S1/M1), with more pronounced effects in the contralateral hemisphere. Furthermore, enhanced functional connectivity between S1/M1 and the medial prefrontal cortex (mPFC) has been reported (Peng et al., 2019).

It has also been suggested that low-frequency TENS produces a more widespread (diffuse) analgesic effect, whereas high-frequency TENS tends to produce a more localized (homotopic) effect. These findings indicate that TENS parameters influence not only the magnitude of analgesia but also the spatial distribution of its effects within the central nervous system (Peng et al., 2019).

2.6. Biophysical Basis of NMES Applications

NMES is an electrotherapy modality that induces muscle contraction by electrically stimulating motor neurons or, in certain conditions, directly activating muscle fibers. In healthy muscle, stimulation is primarily mediated through motor nerve fibers, whereas in cases of denervation, direct excitation of muscle fibers may also occur (Kazak, 2023).

In NMES applications, a frequency range of approximately 20–50 Hz is commonly used, within which tetanic muscle contractions can be achieved. Clinically, NMES is applied to increase muscle strength, reduce muscle atrophy, and support functional mobility (Duymaz, 2018; Kazak, 2023).

The biophysical response of muscle tissue to NMES is dependent on several parameters, including frequency, pulse duration (pulse width), current intensity, and electrode placement. Increasing stimulation duration contributes to the recruitment of a greater number of motor units, while placement of the electrodes over the motor point is associated with higher torque production (Doucet et al., 2012). These findings indicate that parameter selection plays a critical role in both strength development and the onset of fatigue.

Motor unit recruitment during NMES does not strictly follow the physiological “size principle” observed in voluntary contractions. Furthermore, there is limited evidence supporting the reverse recruitment hypothesis. Instead, NMES-induced activation is characterized as non-selective, spatially fixed, and temporally synchronous (Bickel et al., 2011). Due to this constant activation pattern, there is no rotation among active muscle fibers, which may contribute to the earlier onset of neuromuscular fatigue (Doucet et al., 2012; Maffiuletti et al., 2013).

Beyond mechanical muscle contraction, NMES also influences muscle metabolism. Short-duration NMES applied to multiple muscle groups has been shown to significantly increase muscle oxygen consumption (mVO_2). This increase is more pronounced at the local muscle level, although acute elevations in heart rate and whole-body oxygen consumption have also been observed. These findings suggest that NMES can elicit acute physiological responses similar to those seen during exercise (Rege et al., 2026). Even low-frequency (6 Hz) twitch-type stimulation that does not produce tetanic contractions has been reported to induce meaningful metabolic responses (Rege et al., 2026).

Recent review studies have also suggested that NMES does not only induce muscle contraction but may also influence molecular regulatory mechanisms at the cellular level. In this context, NMES has been shown to alter the expression of microRNAs (miRNAs) involved in muscle growth and atrophy. In particular, increases in miR-1 and miR-133 levels have been reported following NMES. Additionally, NMES may influence the balance of muscle protein synthesis and degradation through the miR-486/PTEN/FoxO1 signaling pathway. In animal studies, NMES-unloaded conditions were associated with decreased miR-486 expression and increased levels of muscle atrophy markers such as PTEN, FoxO1, atrogin-1, and MuRF1, whereas NMES intervention reversed these changes (Dickerson et al., 2026). These findings suggest that NMES may be effective in preventing muscle atrophy not only through mechanical activation but also via cellular and molecular regulatory mechanisms.

3. Comparison of the Biophysical Aspects of TENS and NMES Applications

TENS and NMES differ significantly in terms of their biophysical effects and the target tissues they engage. These differences are closely related to the neurophysiological responses induced by electrical stimulation as well as the physiological and biomechanical interactions occurring at the tissue level (Doucet et al., 2012; Kazak, 2023). In addition, the characteristics of stimulation parameters used in each modality play a decisive role in the duration, distribution, and clinical outcomes of the elicited effects (Duymaz, 2018; Özer, 2023). Therefore, the comparison between TENS and NMES should be addressed through a multi-layered biophysical framework rather than a single-level perspective.

3.1. Comparison in Terms of Activated Fiber Types

The primary goal of TENS is to achieve pain modulation through the activation of sensory nerve fibers. In this approach, stimulation of large-diameter myelinated A-beta fibers inhibits pain transmission at the spinal cord level, resulting in a reduction in pain perception. In low-frequency TENS applications, activation of smaller afferent fibers is associated with increased endogenous opioid release, contributing further to analgesic effects (Özen and Koç, 2023; Özer, 2023; Kazak, 2023). Moreover, TENS has been shown to modulate pain not only at the segmental level but also through supraspinal mechanisms via afferent pathways (Johnson, 2007; Peng et al., 2019). These findings indicate that the analgesic effects of TENS are not limited to local mechanisms but involve broader neuromodulatory systems (Johnson, 2007).

In contrast, NMES primarily targets motor neurons or directly stimulates muscle fibers to produce muscle contraction. The applied current induces depolarization in motor nerve fibers, initiating muscle contraction. In innervated muscle, contraction is typically mediated via motor nerves, whereas in denervated conditions, direct muscle fiber stimulation may also occur (Duymaz, 2018; Kazak, 2023). However, muscle contractions induced by NMES do not always follow the physiological “size principle” of motor unit recruitment. Instead, a more synchronous and non-selective activation pattern is observed (Doucet et al., 2012; Maffiuletti et al., 2013). This synchronized activation leads to repeated recruitment of the same motor units, which may contribute to earlier onset of muscle fatigue (Bickel et al., 2011; Doucet et al., 2012).

In this context, it can be stated that TENS primarily activates sensory pathways to induce neurophysiological mechanisms of pain modulation, whereas NMES directly stimulates the motor system to produce muscle contraction, resulting in mechanical loading and increased metabolic activity.

3.2. Comparison of Biophysical Effects of Stimulation Parameters

The electrical stimulation parameters used in TENS and NMES play a fundamental role in determining the resulting biophysical response. In TENS applications, low-amplitude currents are generally preferred and applied at intensities that do not induce muscle contraction. TENS operates across a wide frequency spectrum, where low-frequency stimulation (≤ 10 Hz) is primarily associated with endogenous opioid-mediated mechanisms, while high-frequency stimulation (> 50 Hz) contributes to the inhibition of pain transmission at the spinal level (Özen and Koç, 2023; Özer, 2023; Kazak, 2023).

In NMES applications, since the primary objective is to elicit muscle contraction, current amplitude is increased until a visible contraction is achieved. Frequencies are typically selected within the 20–50 Hz range, while on–off cycles are adjusted to minimize muscle fatigue (Duymaz, 2018; Kazak, 2023). Additional parameters such as waveform, pulse duration, and rise–fall times also influence the biophysical response of the muscle. In this context, TENS parameters are primarily configured to target sensory perception and analgesic effects, whereas NMES parameters are designed to produce muscle contraction, motor activation, and functional output.

3.3. Comparison of Physiological Responses

The primary physiological response induced by TENS is analgesia. This effect is mediated through multiple mechanisms, including segmental inhibition of pain transmission, increased endogenous opioid release, local vasodilation, and stimulation of acupuncture-related points (Özen and Koç, 2023; Özer, 2023; Kazak, 2023). In TENS applications, no visible muscle contraction is typically observed, as sensory-level stimulation is prioritized for pain modulation.

In contrast, the primary physiological response of NMES is muscle contraction. Electrically induced contractions are used to enhance muscle strength, prevent muscle atrophy, support joint range of motion, and improve functional movement capacity. NMES has also been reported to reduce spasticity and improve functional outcomes in certain clinical populations (Duymaz, 2018; Kazak, 2023). Additionally, NMES can increase muscle oxygen consumption and produce acute physiological responses similar to exercise (Rege et al., 2026). In conclusion, although TENS and NMES are based on similar biophysical principles, they exhibit clear differences in terms of target tissues, applied parameters, and resulting physiological responses.

4. Clinical Application of Electrical Stimulation

4.1. Clinical Use of TENS Applications

The stimulation parameters used in TENS applications play a crucial role in the resulting biophysical effects. The main parameter ranges reported in the literature are summarized in Table 1.

Table 1. Electrical Parameters of TENS and Their Clinical Effects

Parameter	Range / Feature	Clinical Significance
Stimulation amplitude	Based on patient tolerance	Determines fiber recruitment level
Stimulation frequency	10–200 Hz	Supports A-beta fiber activation and analgesic mechanisms
Low-frequency TENS	<10 Hz	May activate endogenous opioid system
High-frequency TENS	>50 Hz	May enhance central nociceptive inhibition
Pulse duration	50–200 μ s	Enables selective nerve fiber activation
Short pulse duration	50–100 μ s	Promotes activation of large-diameter fibers
Long pulse duration	>200 μ s	Activates smaller diameter fibers at lower amplitudes
Stimulation pattern	Continuous, burst, modulated	Influences adaptation and patient comfort

The data were compiled based on the mechanisms and clinical application principles of TENS reported by Johnson (2007).

TENS parameters are the primary determinants of both the magnitude of analgesic effects and the underlying neurophysiological mechanisms through which these effects are produced. Amplitude is adjusted according to patient tolerance and directly influences afferent fiber recruitment. Frequency determines the balance between segmental gate control mechanisms and supraspinal descending pain inhibition pathways. Pulse duration shapes the selective activation of different nerve fiber populations, thereby modulating the sensory response profile. The stimulation pattern (e.g., continuous, burst, or modulated) reduces neural adaptation and supports both the continuity of analgesic effects and patient comfort. Overall, the clinical efficacy of TENS emerges from the integrated neurophysiological response generated by the combined interaction of these parameters.

In clinical practice, TENS is reported to be used in the management of acute and chronic pain conditions, postoperative pain control, labor and postpartum pain, musculoskeletal pain syndromes, and various neurological disorders (Duymaz, 2018; Kazak, 2023). It is emphasized that TENS parameters should be individualized based on patient comfort and therapeutic response, and in most

cases optimized through a trial-and-adjustment approach (Johnson, 2007). In particular, high-frequency TENS is associated with segmental inhibition of pain transmission, whereas low-frequency stimulation may produce longer-lasting analgesic effects through activation of endogenous opioid systems (Özen and Koç, 2023; Özer, 2023). Electrode placement is also considered a critical factor, with electrodes typically positioned over the painful area, over relevant dermatomes, or along peripheral nerve pathways (Howarth, 2011).

Table 2 presents data from a recent large-scale meta-analysis evaluating the analgesic effectiveness of TENS across different comparison groups. Overall, TENS demonstrates statistically significant and generally moderate to large pain-relieving effects when compared with placebo, no treatment, standard care, exercise/physiotherapy, and pharmacological interventions. The effect size is particularly pronounced in placebo-controlled studies (SMD: -0.96), and becomes even stronger in studies with low risk of bias (SMD: -1.27), supporting the robustness of its analgesic efficacy (Johnson et al., 2022).

However, when studies with high or unclear risk of bias are included, the magnitude of the effect decreases slightly, although it remains statistically significant. Compared with no treatment and standard care, TENS shows clear superiority, providing a moderate additional benefit especially over standard care (SMD: -0.72). When compared with exercise and physiotherapy, the effect is smaller but still favors TENS, showing a small-to-moderate advantage (SMD: -0.46). Similarly, when compared with pharmacological treatment, TENS demonstrates a meaningful analgesic effect (SMD: -0.82) (Johnson et al., 2022).

Table 2. Evaluation of the Analgesic Effectiveness of TENS Compared with Different Control Groups

Comparison	Number of Studies	Participants	Heterogeneity (I ²)	SMD (95% CI)
TENS vs Placebo	91	4841	88%	-0.96 [-1.14, -0.78]
TENS vs Low risk of bias	15	1104	93%	-1.27 [-1.77, -0.77]
TENS vs High/unclear risk of bias	76	3737	86%	-0.89 [-1.08, -0.70]
TENS vs No treatment	10	602	76%	-0.82 [-1.18, -0.46]
TENS vs Standard care	61	3155	88%	-0.72 [-0.95, -0.50]
TENS vs Exercise/Physiotherapy	25	1114	88%	-0.46 [-0.84, -0.09]

TENS vs Pharmacological treatment	27	1420	86%	-0.82 [-1.13, -0.51]
High vs Low frequency TENS	13	468	39%	-0.19 [-0.43, 0.06]

Data in the table are based on findings reported by Johnson (2022). I^2 : Heterogeneity index; SMD: Standardized mean difference; CI: Confidence interval.

On the other hand, no statistically significant difference was observed between high- and low-frequency TENS applications (SMD: -0.19), suggesting that the analgesic effectiveness may be more closely related to the general neuromodulatory mechanisms of TENS rather than frequency-specific differences (Table 2). Overall, TENS demonstrates a consistent pain-reducing effect across different clinical comparison groups; however, the high heterogeneity observed among studies indicates that the results should be interpreted with caution.

4.2. NMES uygulamalarının klinik kullanımı

NMES applications rely heavily on specific stimulation parameters, which play a critical role in determining the resulting biophysical effects. The fundamental parameter ranges reported in the literature are summarized in Table 3.

Table 3. Stimulation Parameters Used in NMES Applications and Their Clinical Significance

Parameter	Range Used	Clinical Significance
Frequency	20–120 Hz	Determines the type of contraction and level of tetanic contraction; higher frequencies may produce stronger but more fatiguing contractions.
Pulse width	0.1–0.45 ms	Influences the number of activated nerve fibers and comfort; longer pulse widths may recruit more motor units.
Current intensity	30–120 mA or the maximum tolerable level	Determines the strength of muscle contraction; higher intensities may produce greater motor unit recruitment.
Waveform	Rectangular biphasic, square biphasic, monophasic	Affects patient comfort, current distribution, and motor nerve activation.
Session duration	6–90 min	Determines total stimulation dose; prolonged applications may increase adaptation but also fatigue.

Electrode placement	Motor point or muscle belly	Influences efficiency of muscle activation and stimulation effectiveness.

Data in the table are based on findings reported by Borzuola (2023).

Table 3 shows that the clinical effects of NMES are shaped by a wide range of technical variables. Frequency and pulse width are the primary determinants of motor unit recruitment and contraction characteristics. In particular, increasing frequency facilitates the development of stronger and more tetanic contractions, but it may also accelerate the onset of muscle fatigue. Pulse width, on the other hand, modulates the number of activated nerve fibers and patient comfort, thereby influencing the overall intensity of muscle activation.

Current intensity is one of the most important determinants of contraction force, as higher intensities enable the recruitment of a greater number of motor units. Waveform characteristics influence the distribution of current within the tissue and the nature of the sensory–motor response, thereby affecting both the effectiveness of the intervention and patient tolerance. Electrode placement is a critical factor that directly determines the efficiency of target muscle activation, with optimal positioning resulting in more effective muscle contraction. Session duration determines the total stimulation dose and plays a role in both adaptive responses and the development of fatigue. Overall, the wide variability in these parameters allows NMES to be tailored to individual tolerance levels and therapeutic goals in clinical practice. This combination of parameters directly determines the magnitude of therapeutic effects on muscle strength and muscle mass.

Systematic review findings suggest that NMES may contribute to the prevention of muscle weakness and atrophy, particularly in conditions such as prolonged immobilization and critical illness, thereby supporting functional recovery (Maffiuletti et al., 2013).

In recent years, the combined application of NMES with voluntary muscle contraction (superimposed NMES; NMES+) has become an increasingly investigated area in neuromuscular adaptation research. A systematic review by Borzuola et al. (2023) reported that NMES applied during voluntary contractions may provide additional benefits in muscle function and particularly in muscle strength. However, it was emphasized that outcomes vary considerably depending on stimulation protocols, parameter settings, and participant characteristics across studies.

Table 4. Evaluation of NMES and Superimposed NMES (NMES+) Across Different Populations

Study group	Intervention	Duration	Findings
Healthy individuals	NMES + voluntary exercise	4–16 weeks	Increase in muscle strength
Healthy young individuals	NMES + voluntary exercise	4–8 weeks	No significant difference in some studies
Older adults	NMES+	4–12 weeks	Improvement in strength and functional performance
Anterior cruciate ligament injury reconstruction / orthopedic patients	NMES+ rehabilitation	6–12 weeks	Increase in knee muscle strength
Athletes	Whole-body NMES + exercise	5–10 weeks	Improvements in power and performance
Sarcopenic individuals	Whole-body / regional NMES+	4 weeks	Increase in average muscle strength
Muscle hypertrophy/ cross-sectional area	NMES+	Variable	Increase in some studies, no difference in others

Adapted from Borzuola et al. (2023). NMES+: Neuromuscular Electrical Stimulation applied during voluntary contraction.

Table 4 shows that NMES applications, particularly when combined with voluntary muscle contractions (NMES+), tend to produce more pronounced effects on muscle strength. This effect appears to be more evident in populations with limited muscle activation, such as orthopedic patients and older adults. In contrast, several studies report that in healthy young individuals, NMES does not provide additional benefits compared to voluntary exercise alone. Effects on muscle mass are more variable; while some studies report increases, others find no statistically significant differences (Borzuola et al., 2023).

In a randomized controlled trial investigating hemiplegic shoulder pain over a 4-week treatment period, the effects of TENS and NMES were compared. Both interventions were reported to reduce pain; however, the reduction in pain was greater in the NMES group compared to TENS. Despite this, no significant

differences were found between NMES and TENS in terms of shoulder range of motion, upper extremity function, spasticity, or activities of daily living (Zhou et al., 2018).

5. Conclusion

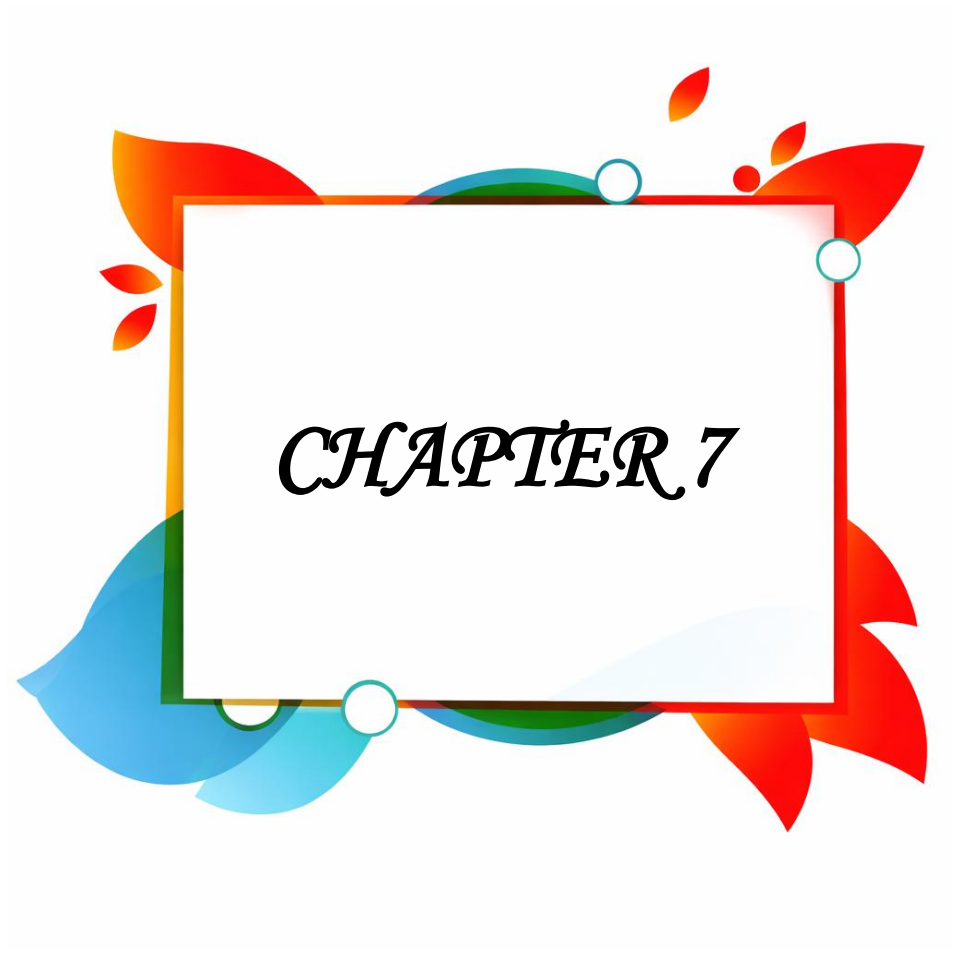
Although TENS and NMES are based on similar electrophysical principles, they differ substantially in terms of target tissues, biophysical responses, and clinical applications. TENS primarily modulates pain by stimulating sensory nerve fibers and activating segmental and supraspinal mechanisms, including the gate control theory, endogenous opioid release, and descending inhibitory pathways. NMES, on the other hand, acts on motor nerves and muscle fibers to induce muscle contractions, thereby contributing to increased muscle strength, prevention of atrophy, and improved functional capacity.

Current literature indicates that TENS can provide significant analgesic effects across various clinical conditions, while NMES offers important benefits for muscle function and strength, particularly in immobilization, neurological, and orthopedic rehabilitation contexts. However, due to heterogeneity in application protocols and variability among patient populations, the effectiveness of electrical stimulation techniques should be interpreted in relation to individual characteristics and therapeutic goals. Accordingly, understanding the biophysical foundations of TENS and NMES is essential for optimizing parameter selection, improving clinical outcomes, and developing personalized electrotherapy approaches.

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CHAPTER 7

Diagnosis and Clinical Management of Acute Diverticulitis

Tarık Sırça¹

1. Introduction and Epidemiology

Colonic diverticulosis is a structural condition characterized by the herniation of the mucosa and submucosa through the muscular layers of the colonic wall, typically at sites where nutrient arteries (vasa recta) penetrate the muscularis propria. While the presence of diverticula (diverticulosis) is largely asymptomatic, approximately 10% to 25% of individuals with diverticulosis will develop acute diverticulitis—an inflammatory complication that represents a significant burden on healthcare systems globally (1,2).

The epidemiology of acute diverticulitis has evolved over recent decades. Historically considered a disease of the elderly, its incidence has been steadily rising in younger populations (individuals under the age of 50). In Western societies, diverticulitis predominantly affects the left colon, particularly the sigmoid colon (approx. 90% of cases). Conversely, in Asian populations, right-sided or cecal diverticulitis is more prevalent and tends to follow a more benign clinical course (3,4).

Risk Factors

Dietary Habits: Low-fiber diets, high intake of red meat, and refined carbohydrates.

Lifestyle: Obesity, physical inactivity, and chronic tobacco use.

Medications: Regular use of nonsteroidal anti-inflammatory drugs (NSAIDs), aspirin, corticosteroids, and opioids significantly elevates the risk of perforation and recurrence.

Genetics: Heritability estimates suggest a substantial genetic susceptibility, affecting colonic wall structural integrity and motility (5).

2. Pathophysiology

The traditional paradigm asserted that mechanical obstruction of a diverticular neck by a fecalith led to increased intraluminal pressure, localized ischemia, and subsequent bacterial proliferation (6). However, contemporary

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pathophysiological models focus more on focal microscopic or macroscopic perforation driven by several key factors:

1. Increased Intraluminal Pressure: Exacerbated by altered colonic motility and low-fiber stools.

2. Ischemic Necrosis: Pressure-induced erosion of the thin diverticular wall adjacent to the vasa recta.

3. Chronic Mucosal Inflammation: Alterations in the colonic microbiome (dysbiosis) and localized immune activation, resembling a localized form of inflammatory bowel disease (IBD).

The extent of the micro-perforation dictates the clinical presentation, ranging from a localized, self-limiting inflammatory process (uncomplicated diverticulitis) to free intraperitoneal rupture with fecal peritonitis (complicated diverticulitis).

3. Clinical Presentation and Diagnostic Evaluation

Clinical Manifestations

The classic presentation of acute left-sided diverticulitis is often termed 'left-sided appendicitis.'

Abdominal Pain: Constant, aching pain localized primarily in the left lower quadrant (LLQ). In right-sided disease, pain localizes to the right lower quadrant (RLQ).

Gastrointestinal Symptoms: Altered bowel habits (constipation is more common than diarrhea), nausea, vomiting, and anorexia.

Constitutional Signs: Low-grade fever, tachycardia, and localized abdominal tenderness.

Peritoneal Signs: Rebound tenderness, guarding, and rigidity indicate localized or generalized peritonitis, signifying macro-perforation or abscess rupture (7).

Laboratory Findings

Leukocytosis: Elevated white blood cell (WBC) count with a left shift is present in the majority of cases.

C-reactive Protein (CRP): A serum CRP level > 50 mg/L is highly suggestive of acute diverticulitis, while levels > 150 mg/L strongly correlate with complicated features (e.g., abscess or perforation).

Urinalysis: Performed to rule out urinary tract infections or colovesical fistulas (indicated by sterile pyuria due to adjacent bladder inflammation).

Imaging Modalities

Diagnostic imaging is mandatory to confirm the diagnosis, rule out mimics (e.g., colon cancer, IBD, pelvic inflammatory disease), and grade severity.

Computed Tomography (CT) Scan: Contrast-enhanced abdominopelvic CT is the gold standard diagnostic modality (sensitivity >95%, specificity >96%). Characteristic CT findings include localized colonic wall thickening (>4 mm), peridiverticular fat stranding, presence of diverticula, and complications such as extraluminal air, fluid collections (abscesses), or fistulous tracts (8).

Ultrasonography (US): A reliable alternative in pregnant patients or resource-limited settings, though highly operator-dependent.

Magnetic Resonance Imaging (MRI): Reserved for pregnant patients or individuals with severe iodinated contrast allergies.

Endoscopy Warning: Optical colonoscopy is strictly contraindicated during the acute phase of suspected or confirmed diverticulitis due to the high risk of converting a micro-perforation into a free, macro-perforation via luminal insufflation.

4. Staging and Classification

The clinical management of acute diverticulitis is directly dictated by its anatomical severity. The Modified Hinchey Classification remains the most widely accepted system for staging complicated diverticulitis on CT imaging.

Hinchey Stage	Pathological Description			CT Findings		
Stage 0	Mild, uncomplicated diverticulitis			Colonic wall thickening, hyperemic changes, mild fat stranding.		
Stage Ia	Confined inflammation/phlegmon		pericolic	Pericolic soft-tissue inflammatory changes; no fluid collection.		
Stage Ib	Pericolic abscess	or	mesenteric	Localized fluid collection < 4 cm or >= 4 cm adjacent to the segment.		
Stage II	Distal (pelvic, retroperitoneal) abscess		intra- or	Abscess distant from the primary inflammatory segment.		
Stage III	Purulent peritonitis			Generalized peritonitis; non-communicating fluid/pus throughout the abdomen.		

Hinchey Stage	Pathological Description	CT Findings
Stage IV	Fecal peritonitis	Frank fecal contamination due to wide-open diverticular perforation.

5. Clinical Management Strategy

Management of Uncomplicated Diverticulitis (Hinchey 0 - Ia)

Recent clinical trials have shifted the treatment paradigm for acute uncomplicated diverticulitis (AUD) from mandatory antibiotic therapy to individualized, conservative management.

Outpatient Management: Immunocompetent patients with mild symptoms, no systemic signs of infection, and reliable social support can be managed safely at home. A clear liquid diet for 24–48 hours is recommended, transitioning to a low-fiber diet as tolerated, and eventually a high-fiber maintenance diet once inflammation completely resolves (9). Recent international guidelines (e.g., AGA, WSES) state that routine antibiotic therapy is unnecessary for uncomplicated diverticulitis in immunocompetent patients. Antibiotics should be reserved for high-risk patients (e.g., immunosuppressed, severe comorbidities, high fever, or leukocytosis).

Inpatient Management: Required for patients unable to tolerate oral intake, those with severe pain requiring intravenous analgesia, or individuals who fail outpatient therapy. These patients are treated with bowel rest (NPO or clear liquids), intravenous fluid resuscitation, and selective or mandatory empirical IV antibiotics.

Management of Complicated Diverticulitis (Hinchey Ib - IV)

Complicated diverticulitis involves an abscess, perforation, fistula, obstruction, or stricture and mandates immediate inpatient admission (10).

Abscess Management (Hinchey Ib and II): Small abscesses (< 3-4 cm) typically resolve with intravenous broad-spectrum antibiotic therapy alone. Larger abscesses ($\geq$ 3-4 cm) require a combination of intravenous antibiotics and CT-guided percutaneous drainage.

Surgical Interventions (Hinchey III and IV): Patients presenting with diffuse purulent (Stage III) or fecal (Stage IV) peritonitis, hemodynamic instability, or failure of non-operative measures require emergency surgery.

Hartmann’s Procedure involves resection of the diseased sigmoid colon, closure of the rectal stump, and creation of an end colostomy. This remains the traditional standard of care for hemodynamically unstable patients. Primary

Anastomosis with or without Diverting Loop Ileostomy is increasingly performed in hemodynamically stable patients with purulent peritonitis (Hinchey III) to avoid a permanent stoma (11).

Empirical Antibiotic Regimens

When antibiotic therapy is indicated, coverage must target Gram-negative rods and anaerobes (e.g., *Escherichia coli* and *Bacteroides fragilis*).

Monotherapy: Ertapenem, Piperacillin/Tazobactam, or Amoxicillin/Clavulanic acid.

Combination Therapy: Ciprofloxacin or Ceftriaxone combined with Metronidazole.

6. Long-Term Follow-Up and Secondary Prevention

Post-Acute Colonoscopy

A complete optical colonoscopy is recommended 6 to 8 weeks following the complete resolution of an episode of acute diverticulitis if the patient has not had a high-quality colonoscopy within the past one to two years (12).

Clinical Pearl: The primary rationale for delayed colonoscopy is not to re-evaluate the diverticula, but to definitively rule out an underlying advanced colonic adenoma or colorectal carcinoma, which can mimic the CT appearance of acute diverticulitis (especially in cases with segmentary colonic wall thickening).

Secondary Lifestyle Modifications

High-Fiber Diet: Gradual increase to 25–35 grams of dietary fiber daily.

Avoidance of Seeds/Nuts: Extensive data have thoroughly debunked the myth that nuts, corn, popcorn, or seeds precipitate diverticulitis; these foods do not need to be avoided.

Physical Activity: Regular vigorous physical exercise lowers recurrence rates.

Weight Reduction: Maintaining a healthy body mass index (BMI).

Smoking Cessation: Strongly encouraged due to its correlation with perforated disease (13).

Elective Resection

The historical rule of performing prophylactic elective sigmoid resection after two episodes of uncomplicated diverticulitis has been abandoned (14). Today, the decision to undergo elective surgery is highly individualized based on the frequency and severity of recurrences, persistent chronic symptoms (smoldering

diverticulitis), the impact on the patient's quality of life, and individual surgical risk profiles.

The standard of care for elective sigmoidectomy is a minimally invasive approach, utilizing either conventional laparoscopy or robotic-assisted surgery. Multiple randomized controlled trials have confirmed that compared to traditional open laparotomy, minimally invasive techniques offer clear advantages:

- Significantly lower rates of surgical site infections (SSI) and incisional hernias.
- Marked reduction in postoperative pain and narcotic requirements.
- Accelerated return of bowel function (peristalsis) and shorter hospital stays (often 1 to 3 days).
- Reduced long-term adhesion formation, protecting against future small bowel obstructions.

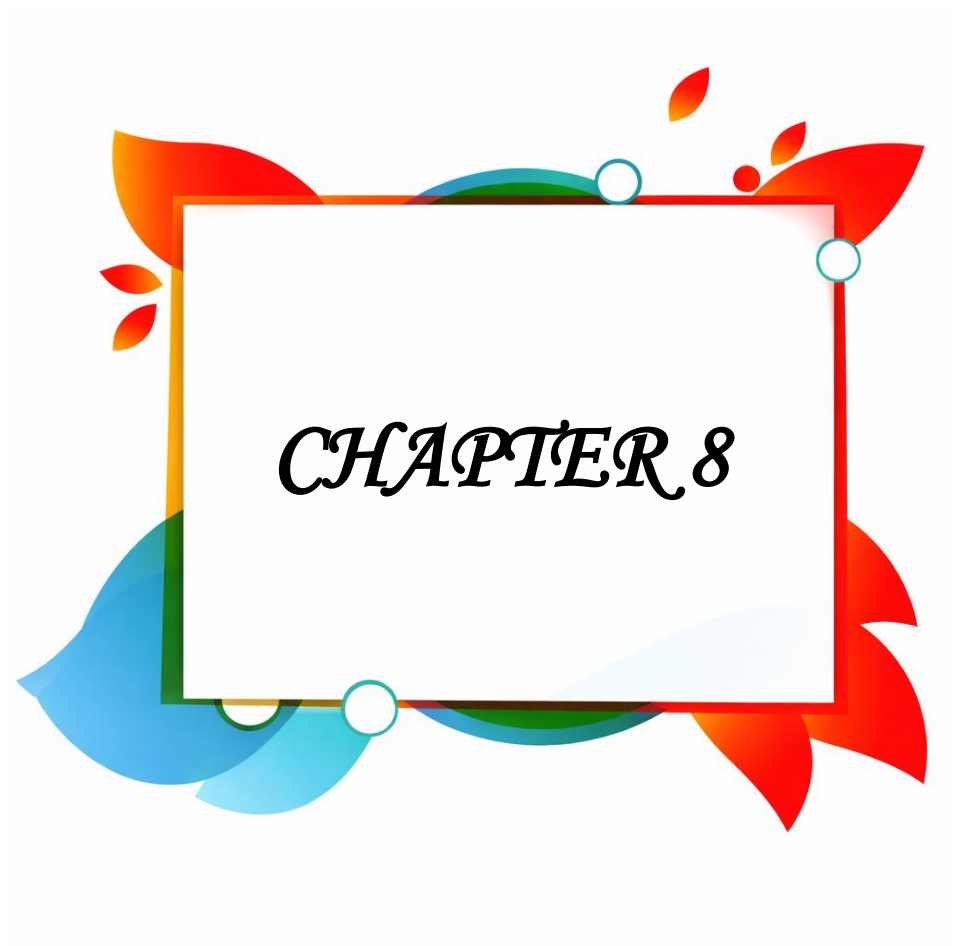
Intestinal continuity is restored using an intra-abdominal stapled anastomosis (typically a side-to-end or end-to-end colorectal anastomosis). Prior to firing the stapler, the surgeon must ensure that the mobilized descending colon reaches the rectal stump completely free of tension and that its mesenteric blood supply is pristine.

Once the anastomosis is constructed, a formal leak test (air-insufflation test) is performed. The pelvis is filled with sterile saline, and the rectum is gently insufflated with air via a sigmoidoscope or bulb syringe while the proximal colon is occluded. The absence of air bubbles confirms a mechanically sound, airtight seal. If bubbles are detected, the site must be immediately reinforced with surgical sutures or, if necessary, dismantled and reconstructed to prevent a postoperative anastomotic leak (15).

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Clinical Approach to Thyroid Nodules

Alper Çalışkan¹

1: Introduction and Epidemiology

Thyroid nodules are discrete lesions within the thyroid gland that are radiologically distinct from the surrounding thyroid parenchyma. They are exceptionally common in clinical practice. The detection rate of thyroid nodules has increased dramatically over the past few decades, primarily due to the widespread use of high-resolution ultrasonography (US) and other incidental imaging modalities like computed tomography (CT), magnetic resonance imaging (MRI), and positron emission tomography (PET) scans.

Palpable thyroid nodules occur in approximately 4% to 7% of the adult population. However, ultrasound detects nodules in up to 20% to 67% of randomly selected individuals, particularly in women, older adults, and individuals residing in iodine-deficient regions (1,2).

The primary clinical objective when evaluating a thyroid nodule is a balanced approach: accurately identifying the minority of nodules that harbor clinically significant malignancy (roughly 5% to 15% depending on age, sex, radiation history, and family history) while avoiding unnecessary invasive procedures, surgeries, and psychological distress for the vast majority of patients with benign nodules (3).

2: Clinical Evaluation and Risk Stratification

A meticulous clinical history and physical examination represent the foundation of thyroid nodule management. While most nodules are asymptomatic, specific clinical red flags significantly increase the suspicion of thyroid cancer.

Clinical Risk Factors for Malignancy

- **History of Radiation:** Childhood neck, head, or upper chest irradiation, or exposure to nuclear fallout.
- **Family History:** First-degree relatives with medullary thyroid carcinoma (MTC), papillary thyroid carcinoma, or syndromic disorders like Multiple Endocrine Neoplasia type 2 (MEN 2), Cowden syndrome, or Familial Adenomatous Polyposis (FAP).
- **Demographics:** Male sex, extremes of age (less than 20 or greater than 70 years old).

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- **Symptomatology:** Rapid nodule growth, persistent hoarseness or dysphonia (suggesting recurrent laryngeal nerve palsy), dysphagia, or dyspnea.
- **Physical Examination Findings:** A firm, hard, or fixed nodule; associated ipsilateral cervical lymphadenopathy.

Initial Laboratory Assessment

The evaluation of any thyroid nodule must begin with a serum Thyroid-Stimulating Hormone (TSH) measurement.

1. **Low TSH:** If serum TSH is suppressed, the next step is a Thyroid Radionuclide Scintigraphy (using technetium-99m or iodine-123). 'Hot' (hyperfunctioning) nodules are almost always benign and rarely require fine-needle aspiration (FNA).

2. **Normal or Elevated TSH:** If TSH is normal or high, the nodule is considered 'non-functioning,' and further evaluation depends entirely on its sonographic appearance and size.

3. **Serum Calcitonin:** Routine measurement remains controversial but is highly recommended if a family history of MTC or MEN 2 is present (4.5).

3: Ultrasonography and TIRADS Classification

High-resolution thyroid ultrasound is the most sensitive and cost-effective modality for characterizing thyroid nodules. Ultrasound features are used to estimate the risk of malignancy and guide the decision for FNA.

High-Risk Sonographic Features

- **Microcalcifications:** Tiny, punctate echogenic foci without acoustic shadowing.
- **Hypoechoogenicity:** The nodule is darker than the surrounding thyroid tissue or neck musculature.
- **Irregular Margins:** Infiltrative, microlobulated, or spiculed borders.
- **Taller-than-Wide Shape:** An anteroposterior diameter greater than the transverse diameter (highly specific for papillary carcinoma).
- **Extrathyroidal Extension:** Disruption of the thyroid capsule.
- **Interrupted Rim Calcifications:** Especially with small soft tissue components extruding through the calcification.

To standardize risk stratification, societies use point-based or pattern-based systems. The American College of Radiology (ACR) TI-RADS system scores nodules across five categories (composition, echogenicity, shape, margin, and echogenic foci) to determine management thresholds (6):

ACR Category	TI-RADS	Risk Classification	FNA Threshold	Size	Follow-Up Threshold	US
TR1		Benign	No FNA		No follow-up	
TR2		Not Suspicious	No FNA		No follow-up	
TR3		Mildly Suspicious	≥ 2.5 cm		≥ 1.5 cm	
TR4		Moderately Suspicious	≥ 1.5 cm		≥ 1.0 cm	
TR5		Highly Suspicious	≥ 1.0 cm		≥ 0.5 cm	

4: Fine-Needle Aspiration (FNA) and Cytopathology

Fine-needle aspiration is the gold standard for differentiating benign from malignant thyroid lesions. Cytological results are globally categorized according to The Bethesda System for Reporting Thyroid Cytopathology.

The Bethesda Classification System

- **Bethesda I: Non-diagnostic or Unsatisfactory (Risk of Malignancy: 5-10%)**

Management: Repeat ultrasound-guided FNA in 4 to 12 weeks.

- **Bethesda II: Benign (Risk of Malignancy: 0-3%)**

Management: Clinical and sonographic follow-up.

- **Bethesda III: Atypia of Undetermined Significance / Follicular Lesion of Undetermined Significance (AUS/FLUS) (Risk of Malignancy: 10-30%)**

Management: Repeat FNA, molecular testing, or diagnostic lobectomy.

- **Bethesda IV: Follicular Neoplasm / Suspicious for Follicular Neoplasm (FN/SFN) (Risk of Malignancy: 25-40%)**

Management: Molecular testing or diagnostic lobectomy.

- **Bethesda V: Suspicious for Malignancy (Risk of Malignancy: 50-75%)**

Management: Near-total thyroidectomy or lobectomy.

- **Bethesda VI: Malignant (Risk of Malignancy: 97-99%)**

Management: Therapeutic surgical resection (7).

The Role of Molecular Testing

For indeterminate cytology (Bethesda III and IV), molecular diagnostic panels (such as Afirma GSC or ThyroSeq v3) have revolutionized management. These panels look for genetic mutations (BRAF V600E, RAS, RET/PTC) or gene expression profiles. A test with high negative predictive value (NPV) can rule out malignancy, sparing the patient from diagnostic surgeries (8).

5: Management and Therapeutic Paradigms

1. Management of Benign Nodules (Bethesda II)

Benign nodules require periodic monitoring. Initial follow-up ultrasound is typically performed 6 to 12 months after the initial evaluation. If the size remains stable, the interval can be lengthened to 2 to 5 years. Routine intervention is not indicated unless the nodule causes structural symptoms (dysphagia, airway compression) or cosmetic concerns, in which case surgery or minimally invasive thermal therapies (e.g., Radiofrequency Ablation - RFA) are utilized (9,10).

2. Management of Indeterminate Nodules (Bethesda III & IV)

Management must be highly individualized using shared decision-making. Options include molecular testing, active monitoring if clinical and ultrasound risk is low, or diagnostic thyroid lobectomy.

3. Management of Malignant and Suspicious Nodules (Bethesda V & VI)

Surgical resection remains the primary treatment for malignant nodules.

- **Thyroid Lobectomy:** Increasingly favored for unifocal, low-risk papillary thyroid carcinomas measuring less than 4 cm without evidence of extrathyroidal extension or lymph node metastasis.
- **Total Thyroidectomy:** Indicated for tumors greater than 4 cm, bilateral disease, presence of gross extrathyroidal extension, or known distant metastases.
- **Active Surveillance:** An emerging, safe alternative for low-risk papillary microcarcinomas (less than 1 cm) without aggressive clinical or imaging features, managed with serial ultrasounds every 6 months.

4. Postoperative Management and Surgical Complications

Surgical complications can significantly impact patient quality of life and require swift, evidence-based management:

- **Hypocalcemia:** Transient hypocalcemia occurs in up to 30% of total thyroidectomy cases due to

parathyroid stunning, while permanent hypoparathyroidism (<2%) requires lifelong calcium and calcitriol. Serum calcium and intact PTH monitoring guide early postoperative supplementation (11).

- **Recurrent Laryngeal Nerve Injury:** Unilateral RLN injury presents as postoperative

hoarseness, breathiness, or loss of high-pitched voice tones. Bilateral RLN injury is a life-

threatening emergency presenting with acute airway obstruction and stridor upon extubation,

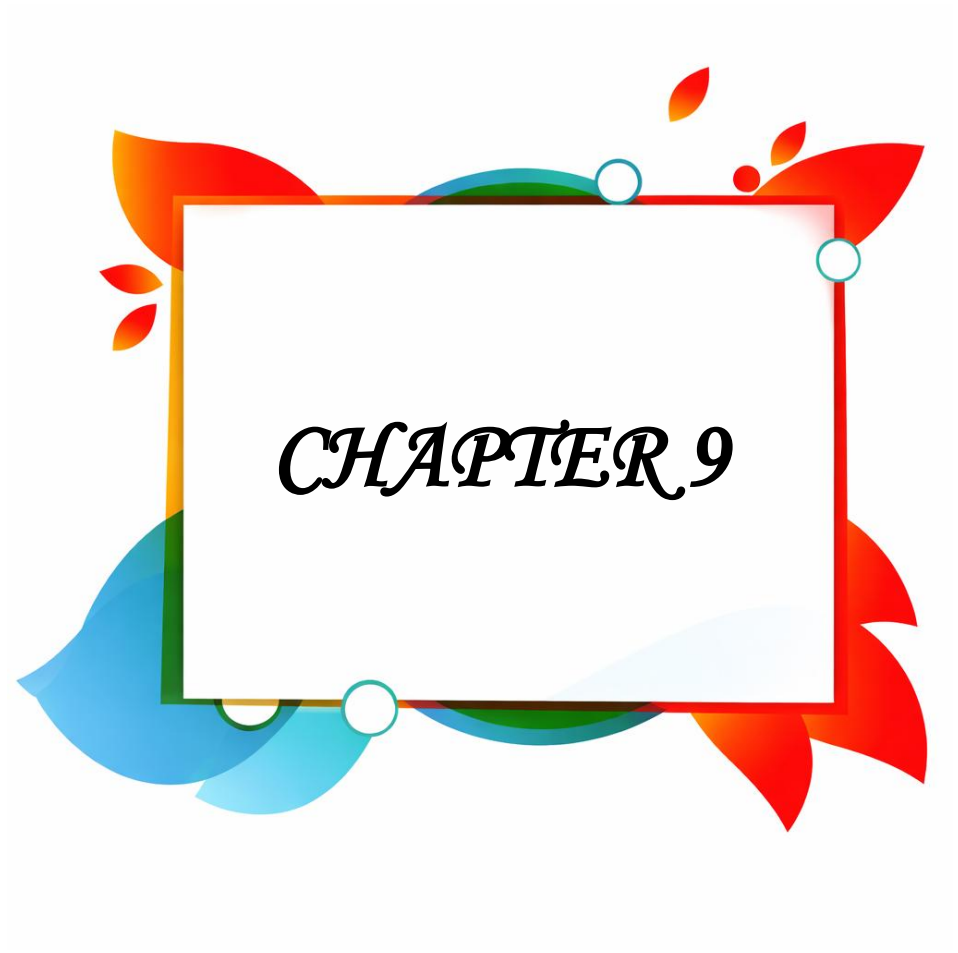
frequently requiring urgent tracheostomy or cordectomy.

Neck Hematoma: Postoperative bleeding leading to a neck hematoma is a rare (<1%) but critical emergency. Rapid expansion can compress the airway, necessitating immediate bedside decompression (opening the wound incisions) and emergent surgical re-exploration for hemostasis (12).

Hormone Replacement and TSH Suppression: Patients undergoing total thyroidectomy require lifelong levothyroxine therapy. Weight-based dosing (approx. 1.6 mcg/kg/day) is initiated, with subsequent serum TSH monitoring at 6-8 weeks. For low-risk differentiated cancers, TSH is maintained in the low-normal range (0.5–2.0 mU/L), while high-risk cancers require suppressive therapy (<0.1 mU/L).

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Intraoral Maxillary Molar Distalization Methods in Class II Malocclusion Treatment

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INTRODUCTION

Molar distalization, one of the space-creating techniques in orthodontics, is a treatment method used by orthodontists to increase arch length by moving the molars distally. (Cambiano, Janson, Fuziy, Garib, & Lorenzoni, 2017; Sfondrini, Cacciafesta, & Sfondrini, 2002) In orthodontics, maxillary molar distalization has long been successfully used, particularly in cases of Class II malocclusion. This technique is typically employed to create space in order to reduce crowding and excessive overjet. (Almuzian, Alharbi, White, & McIntyre, 2017) The greatest advantage of molar distalization is that it is a conservative treatment that eliminates the need for tooth extraction to create space. (Anasart, Pattarahirun, Suzuki, Suzuki, & Sukjamsri, 2019; Patil et al., 2016)

The mechanics of molar distalization consist of two categories: extraoral and intraoral methods. Extraoral methods include appliances such as cervical, occipital, and combined headgear, while intraoral distalization methods include magnetic appliances, nickel-titanium (Ni-Ti) springs, and appliances such as the Pendulum, Jones Jig, First Class, Distal Jet, and Carriere Motion.

Intraoral molar distalization methods can be categorized into two groups: intramaxillary and intermaxillary methods. In intramaxillary intraoral molar distalization methods, the anchorage required to achieve molar distalization is provided by the anterior teeth of the upper jaw and/or implants. In intermaxillary intraoral distalization methods, however, the anchorage is provided by dental structures in the lower jaw.

Extraoral appliances depend primarily on patient compliance and have led to the preference for intraoral distalization appliances due to aesthetic concerns. Intraoral appliances are more readily accepted by patients and also allow orthodontists to exercise greater control over the teeth. For this reason, intraoral appliances have become the more frequently chosen option. (Jeckel & Rakosi, 1991)

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1. Intermaxillary Appliances

1.1. Herbst Appliance

The Herbst appliance is a fixed functional appliance introduced by Herbst in the early 1900s. Pancherz modified the Herbst appliance, reintroducing it as a banded device, and highlighted its potential to stimulate mandibular condylar growth. (Pancherz, 1985) The Herbst appliance has a restrictive effect on maxillary growth and a stimulating effect on mandibular growth. It has been reported that the sagittal molar movement achieved with this appliance results from skeletal changes in 43% of cases and dentoalveolar changes in 57% of cases. (Pancherz, 1979) Dentoalveolar changes include proclination of the lower incisors and distalization of the maxillary molars. (Kurt & Sakin, 2010)

1.2. Jasper Jumper Appliance

The Jasper Jumper appliance is a semi-elastic, fixed functional orthodontic device consisting of bilateral flexible springs that apply continuous force to both molars. The appliance works by utilizing the patient's growth potential to advance the mandible, thereby correcting Class II malocclusions in a relatively short period of time. It is easy to use because it allows for free lateral jaw movement and is generally well tolerated by patients. (Küçükkeleş, İlhan, & Orgun, 2007)

Various studies have reported that the effects of the appliance are largely dentoalveolar in nature. These changes include the distal inclination and relative intrusion of the upper molars, as well as the buccal inclination and intrusion of the lower incisors. (Cope, Buschang, Cope, Parker, & Blackwood III, 1994) Furthermore, it is stated that the Jasper Jumper appliance's effect of restricting maxillary growth is more pronounced than the skeletal effect it produces in the mandible. (Küçükkeleş et al., 2007)

1.3. Forsus Appliance

The Forsus Fatigue Resistant Device (FRD) is a semi-rigid telescopic functional appliance compatible with fixed orthodontic treatments; it is used to treat Class II malocclusions by positioning the mandible forward. (G. Jones, Buschang, Kim, & Oliver, 2008) It shows its effect by applying continuous force thanks to the superelastic NiTi spring system.

The literature indicates that the effects of the Forsus appliance are largely of dentoalveolar origin. Sagittal correction is achieved through dental movements such as distalization of the upper molars, mesialization of the lower molars, retrusion of the upper incisors, and protrusion of the lower incisors. (Heinig & G, 2001) Skeletal effects, however, are generally limited; some studies have observed restricted maxillary growth and a slight increase in mandibular length.

(Franchi et al., 2011; Mahamad, Neela, Mascarenhas, & Akhter Husain, 2012; Oztoprak, Nalbantgil, Uyanlar, & Arun, 2012)

1.4. Wilson 3D Bimetric Molar Distalization Arch

The Wilson 3D Bimetric Distalization Arch is a fixed molar distalization system that, although an intraoral method, requires patient compliance. (Kurt & Sakin, 2010) In this technique developed by Wilson and Wilson, open-coil springs made of Elgiloy wire are used as the force source, and the system is supported by Class II elastics extending from the lower first molars to the upper canines. The use of elastics is recommended, and this approach is defined as the “elastic load reduction” principle. (Wilson & Wilson RC, 1984, 1987)

Various studies have indicated that the effects of the appliance are largely dentoalveolar in nature. In their study, Wilson and Wilson reported that parallel distal movement occurred in the upper first molars, while no changes were observed in the lower incisors. (Wilson & Wilson RC, 1984) However, some studies have identified distal tilting in the upper first molars, along with protrusion of the upper and lower incisors, an increase in overjet, and mesial movement of the lower molars. (Küçükkeleş & Doganay, 1994; Muse, Fillman, & Mitchell, 1993; Üçem, Yüksel, Okay, & Gülsen, 2000)

1.5. Open Coil Springs

Open coil springs are spring systems that transmit a thrust force in both directions from the center when activated and are widely used in orthodontic practice. It has been noted that as the number of wires in the spring increases, tooth movement can be achieved with lighter and more effective forces. (Chaconas, Caputo, & Harvey, 1984)

Gianelly et al., who developed this force system, used NiTi superelastic open-coil springs positioned between the first premolar and the first molar on a passive 0.016“ × 0.022” archwire and activated to generate 100 g of force. (Gianelly, Bednar, & Dietz, 1991; Gianelly, Vaitaa, & Thomas, 1989) A Nance appliance has been incorporated into the system to preserve the anterior anchorage. For clinicians seeking to further strengthen anchorage support, an option is available for a 0.018" uprighting spring placed in the vertical slot of the first premolar bracket. This spring, which directs the premolar crown distally, is used in conjunction with Class II elastics. (Kurt & Sakin, 2010) Researchers have reported that they achieved distalization of the upper first molar at a rate of 1–1.5 mm per month using this protocol. (Gianelly et al., 1991)

In their studies, Erverdi et al. also reported that open-coil nitinol springs provide greater distalization compared to magnets. (Erverdi, Koyutürk, & Küçükkeleş, 1997)

1.6. Carriere Motion 3D Appliance

The Carriere Motion 3D Appliance, developed by Luis Carriere in 2004, is an intraoral intermaxillary Class II corrector that requires patient compliance. (Carriere, 2004) The fundamental principle of treatment is based on establishing a Class I molar relationship before proceeding to the leveling and alignment phase. (Kim-Berman, McNamara, Lints, McMullen, & Franchi, 2019) It is recognized as an effective treatment option for patients in the growth phase, and this effectiveness is based on a combination of mesial movement of the mandibular molars, distal rotation of the maxillary molars, and anterior displacement of the mandible. (Biggs et al., 2023)

The appliance consists of two rigid bars anchored bilaterally to the maxillary canines and first molars. Intermaxillary elastics are attached to the mandibular molars; for anchorage, an Essix plate, a lower lingual arch, or temporary anchorage devices may be used. (Sandifer, English, Colville, Gallerano, & Akyalcin, 2014) Treatment involves wearing Class II elastics with a tension of 6–8 oz for 22 hours a day, and in compliant patients, Phase I treatment is typically completed within 5–8 months.

The changes resulting from the treatment were largely dentoalveolar in nature; distal tipping and rotation were observed in the maxillary molars and canines, while mesial movement was observed in the mandibular molars. (Areepong, Kim, Oliver, & Ueno, 2020) It has been reported that the use of mini-implants offers advantages in preventing proclination of the mandibular incisors. (Fouda, Attia, Abouelezz, Abd El-Ghafour, & Aboufotouh, 2022)

2. Intramaxillary Tooth-Supported Appliances

2.1. Transpalatal Arch

The transpalatal arch (TPA) is an appliance consisting of a 0.9 mm thick rigid stainless steel wire that allows for the lengthening of the arch through rotation, expansion, and distalization, and does not require patient compliance. Haas and Cisneros have noted that by applying a distalizing force equivalent to 1/4 to 1/8 of the headgear force using the TPA, distobuccal rotation and distal tipping of the maxillary first molars can be achieved, resulting in limited distalization. (Haas & Cisneros, 2000)

Given this limitation, it is emphasized that the appliance is particularly suitable for use in cases of unilateral Class II malocclusion or in cases where there is significant mesiobuccal rotation of the molars. (Mandurino & Balducci, 2001; Sfondrini et al., 2002)

2.2. Pendulum Appliance

The pendulum appliance, introduced by Hilgers, is a fixed molar distalization appliance consisting of 0.032-inch-thick TMA springs that transmit a gentle,

continuous force distally to the maxillary first molars via a modified Nance button anchored to the first and second premolars with occlusal supports. (Hilgers, 1992) When the springs are activated to 90° and placed in the mouth, 30% of the activation is lost when they are seated in the palatal sheaths, and a force of approximately 230 grams is generated on one side with a net activation of 60°. Activation is performed every 3 weeks, and with this protocol, 5 mm of molar distalization is achieved in 3–4 months.

In the studies conducted, distalization and distal tipping of the upper first molars, mesialization and mesial tipping of the first premolars, and an increase in overjet due to labial movement of the incisors were observed. (Bussick & McNamara, 2000; Byloff & Darendeliler, 1997; Ghosh & Nanda, 1996) In their study comparing the Pendex appliance—created by adding an expansion screw to the acrylic portion of the pendulum appliance—with cervical headgear, Taner et al. found that the treatment duration was shorter in the Pendex group, but that inclination of the anterior teeth was observed only in this group. (Taner, Yukay, Pehlivanoglu, & Çakırer, 2003) It has been reported that the apparatus has been modified and used by many researchers. (Bussick & McNamara, 2000; Scuzzo, 1999; Kinzinger, Fuhrmann, Gross, & Diedrich, 2000)

2.3. Jones Jig Appliance

The Jones Jig was introduced by Jones and White as a system consisting of a NiTi open-coil spring adapted to a thick segmental archwire. (Jones & White, 1992) In this system, which utilizes a modified Nance appliance for anchorage, a force of 70–75 grams is applied in 1–5 mm increments, and the adjustment is repeated every 4–5 weeks.

In the studies conducted, distalization, distal tipping, distopalatal rotation, and extrusion were observed in the upper first molars; meanwhile, mesial movement and tipping, an increase in overjet, and an increase in lower anterior arch height were reported in the upper second premolars. (Gulati, Kharbanda, & Parkash, 1998; Runge, Martin, & Bukai, 1999) In their study comparing the Jones Jig with cervical headgear, Haydar and Uner found a shorter distalization time and greater distal tilting of the molars in the Jones Jig group; however, they reported a higher incidence of anchorage loss in this group. (Haydar & Uner, 2000) Due to the risk of mandibular rotation associated with molar extrusion in individuals exhibiting excessive vertical growth, careful use of the appliance is recommended in these cases. (Gulati et al., 1998)

2.4. Distal Jet Appliance

The Distal Jet appliance was developed by Carano and Testa to address issues of distal tipping and rotation in intraoral distalization techniques. (Carano, 1996) The appliance uses NiTi open-coil springs that slide along a thick wire; one end of the wire is embedded in the acrylic component of a modified Nance appliance,

while the other end is placed in the palatal tube of the upper first molar to be distalized. By applying force at a point close to the molar center of resistance, it is expected that this method will result in less tipping and more body movement compared to other intraoral distalization techniques. (Sfondrini et al., 2002)

In their study, Ngantung et al. reported that correction of a Class II molar relationship using a distal jet appliance was completed in an average of 6.7 months and that the molars moved in parallel due to the application of force near the center of resistance; however, they emphasized that significant anchorage loss occurred. (Ngantung, Nanda, & Bowman, 2001) Bolla et al. also applied the appliance using fixed appliances and reported that distalization was observed in the molars, mesialization in the premolars, and proclination in the incisors. (Bolla, Muratore, Carano, & Bowman, 2002) In their study comparing the Distal Jet and Pendulum appliances, Chiu et al. found that the group treated with the Pendulum appliance achieved greater distal movement in the molars and experienced less loss of anchorage. (Chiu, McNamara, & Franchi, 2005) Given that loss of anterior anchorage is the main disadvantage of the appliance, its use is recommended with caution in cases of maxillary incisor protrusion, anterior open bite, and a protrusive profile. (Sfondrini et al., 2002)

2.5. First Class Appliance

The First Class appliance was designed by Fortini et al. to minimize the loss of anchorage in the anterior region caused by the Distal Jet appliance. (Fortini, Lupoli, & M Parri, 1999) The appliance consists of two components: the vestibular and the palatal components. The vestibular component includes formative screws, split rings, and retaining screws, while the palatal component is designed as a modified Nance button with a wider profile. NiTi open coil springs, approximately 10 mm in length, are placed between the premolars and molars and serve as the force source.

In their study of 62 individuals with Class II malocclusion, Fortini et al. reported that they achieved an average of 4.8 mm of molar distalization in just 42 days. (Fortini et al., 1999) Another study reported that a bilateral Class II molar relationship was corrected in 2.4 months, with 4 mm of distalization and 4.6° of distal inclination observed in the maxillary first molars. (Fortini, Lupoli, Giuntoli, & Franchi, 2004) However, loss of anchorage was observed in the form of mesialization and tipping of the second premolars, and labial movement and proclination of the maxillary incisors. (Fortini et al., 2004) In their study of patients with Class II malocclusion during the mixed dentition period, Papadopoulos et al. reported that the average treatment duration required to achieve a Class I molar relationship was 17.2 weeks and that a molar distalization of 1 mm per month was achieved. In this study, an average of 4 mm of molar distalization and 8.56° of distal tipping were observed, while no distal rotation was noted; however, anterior anchorage loss in the form of increased overjet,

accompanied by mesial movement and tipping of the first premolars, was observed. (Papadopoulos, Melkos, & Athanasiou, 2010)

3. Intramaxillary Skeletal-Supported Appliances

The most significant limitation of tooth-supported intramaxillary distalization techniques is loss of anchorage. In these techniques, the reactive component of the applied force is transmitted to the maxillary anterior teeth, resulting in loss of anchorage that manifests as mesialization and mesial tipping of the premolars, and protrusion and proclination of the incisors. It has been reported that anchorage loss can account for 24–50% of the total amount of molar distalization. (Sfondrini et al., 2002) To address this issue, temporary anchorage devices (TADs) have begun to be used, which are placed in the bone rather than in dental structures.

In skeletal-supported distalization systems, the reactive component of the force is transmitted to the bone via a titanium screw or plate; this largely eliminates undesirable effects on the anterior teeth. These systems provide reliable anchorage independent of patient cooperation; numerous studies have shown that, compared to conventional methods, loss of anterior anchorage remains at a negligible level. (Kurt & Sakin, 2010) TADs are classified into three groups based on their location: palatal, buccal, and infrazygomatic.

3.1. Palatal Mini-Screws

The palatal region stands out as the most preferred site for mini-screw placement due to its high bone density, low risk of root damage, and large surface area. Mini-screws are typically placed in the anterior palatal region, a few millimeters distal to the midpalatal suture and posterior to the incisive foramen. (Kurt & Sakin, 2010) The placement of implants in the palate for orthodontic anchorage was first introduced by Triaca et al. in 1992. (Triaca, Antonini, & Wintermantel, 1992) Mannchen's case experiences and those of subsequent researchers laid the groundwork for the development of modified appliances supported by mini-screws in later years. (Mannchen, 1999)

Another biomechanical advantage of the palatal mini-screw placement is that the applied force vector is positioned closer to the center of resistance of the molar than it is to the buccal region; this supports distal body movement while reducing distal tipping.

In the systematic review conducted by Mohamed et al., it was reported that in 85.71% of the 14 studies examined, mini-implants were placed in the paramedian palatal region. This review noted that palatally placed systems can achieve molar distalization of more than 5 mm in premolars and incisors without causing significant side effects. It was reported that molar distalization values ranged from 1.8 mm to 6.4 mm, while distal tipping ranged from 1.65° to 11.3°. (Mohamed et al., 2018)

In the study by Huang et al., which included a subgroup analysis based on miniscrew placement, it was reported that an average of 3.81 mm of distalization was achieved in the palatal miniscrew group; however, molar distal tipping in this group was significantly higher than in other regions. The researchers emphasized that this high tipping value is largely due to the pendulum arm mechanism, noting that applying force to the crown region—which is distant from the center of resistance—increases distal tipping. (Huang, Wang, Lu, Su, & Lei, 2025)

In a systematic review encompassing 11 studies (230 patients, permanent dentition) evaluating distalization achieved exclusively with palatal TAD-supported appliances, Fiorillo et al. reported first molar distalization values ranging from 3.0 to 5.3 mm; in all studies, they observed spontaneous distal movement rather than loss of anchorage in the premolars. Researchers attribute this finding to the fact that the force is directed toward the osseous anchorage devices rather than the teeth, causing the premolars to be pulled distally via transseptal fiber connections. (Fiorillo et al., 2022)

In their study, Ceratti et al. demonstrated the decisive effect of appliance rigidity on tipping. According to their findings, palatal appliances with rigid arms cause less distal tipping than non-rigid (pendulum-type) appliances. However, they noted that there was no significant difference in the degree of distalization between the two groups. (Ceratti et al., 2024)

3.2. Buccal Mini-Screws

The buccal interradicular region requires a technically more challenging placement procedure due to the thin cortical bone layer and proximity to the root. However, mini-screws placed in this region offer a significant clinical advantage because force can be transmitted directly to the archwire without the need for additional connecting elements.

The first studies evaluating the use of buccal interradicular miniscrews for orthodontic distalization were conducted by Park et al. Park et al. achieved 2.5 mm of distalization in all posterior teeth over 17 months by placing micro-screws in the buccal alveolar bone between the second premolar and first molar in the maxilla, using NiTi closed-coil springs applied from the canines to the micro-screws. (Park, Kwon, & Sung, 2004) In their other studies, they reported that distalization occurred in all posterior teeth following an average of 12.3 months of treatment; there was no loss of anchorage in the anterior teeth, and instead, lingual tipping was observed. (Park, Lee, & Kwon, 2005)

The most significant limitation of using buccal interradicular miniscrews is the risk of the screw coming into contact with adjacent tooth roots during the distalization process. For this reason, it is recommended that the screw be placed at an angle of 30–40° to the long axis; with oblique placement, the tip of the screw is directed toward the apical region, which is wider than the roots, and the depth

of penetration into the bone is reduced. (Yoon et al., 2020) Yoon et al. used CBCT simulation to model the screw-to-root distance in molar distalization with a buccal interradicular miniscrew based on screw angle and vertical facial type; they reported that screw repositioning may be necessary in cases where the amount of distalization exceeds the existing interradicular space. Due to this anatomical limitation, they noted that distalization using a buccal interradicular miniscrew is generally suitable for cases of 3 mm or less. (Yoon et al., 2020)

In their study investigating the effect of the number of interradicular mini-screws on distalization, Bechtold et al. divided 25 adult patients into two groups: a single-screw group and a double-screw group. Molar distalization was found to be significantly greater in the double-screw group compared to the single-screw group. In the vertical plane, clockwise rotation of the occlusal plane was observed in the single-screw group, whereas the entire arch exhibited intrusion in the double-screw group, and a significant decrease in the SN-MP angle was noted. (Bechtold, Kim, Choi, Park, & Lee, 2013)

Studies have shown that the buccal interradicular region produces a more limited amount of distalization compared to other placement sites. Bayome et al. reported an average of 2.75 mm of molar distalization in the buccal interradicular TAD group, Ceratti et al. reported 3.23 mm, and Huang et al. reported 2.01 mm. Researchers attribute this limitation to the anatomical proximity of the buccal mini-implants to the roots, which restricts the distance of distalization. (Bayome, Park, Bay, & Kook, 2021), (Ceratti et al., 2024), (Huang et al., 2025)

3.3. Infrazygomatic Crest Mini-Screws

The infrazygomatic crest region corresponds to the anatomical structure where the zygomatic bone meets the maxillary alveolar bone distal to the first molar. The increased cortical bone thickness in this region allows for the reliable achievement of primary stability and the ability to withstand high forces. In this region, which is easily accessible via the buccal approach, mini-screws can transmit direct distalizing forces through elastics or springs attached to the archwire.

In their study, Huang et al. reported that they achieved the highest amount of molar distalization—4.9 mm—in the infrazygomatic mini-screw subgroup compared to the buccal and palatal regions. In the infrazygomatic group, the average molar distal tipping was measured at 4.27°, and the incisor distal movement was -1.27 mm. (Huang et al., 2025)

In their study using infrazygomatic crest mini-implants, Rosa et al. achieved 4 mm of molar distalization, 11.2° of distal tipping, and 1.2 mm of molar intrusion in 25 adolescent patients following an average treatment duration of 7.7 months. In the incisor region, 4.7 mm of retraction and 13.4° of lingual tipping were

observed. In the study, all patients achieved a Class I molar relationship, and no screw failure was reported. (Rosa et al., 2023)

3.4. Modified Appliances with Mini-Screw Support

The modification of conventional intraoral distalization appliances using mini-screws is based on the principle of transferring the anchorage from the tooth to the bone while preserving the biomechanical framework of the existing appliance. In this hybrid approach, the Nance section or the tooth-supported anchorage unit of the appliance is reinforced with a mini-screw, thereby freeing the anterior segment.

Modifications of the pendulum appliance with palatal anchorage are among the earliest attempts in this field. Byloff et al. introduced the Graz implant-supported pendulum (GISP) appliance, which combines the principle of the pendulum appliance with bone anchorage. In this system, the movable pendulum component is mounted on an anchor plate surgically placed in the center of the palate using four mini-screws; TMA springs apply 250 g of force at 45° activation, while NiTi open-coil springs apply 80–100 g of force. (Byloff, Karcher, Clar, & Stoff, 2000) Kircelli et al. achieved 6.4 mm of distal movement in 10 patients following seven months of treatment using a bone-anchored pendulum appliance (BAPA) with an osseointegrated implant placed 7–8 mm posterior to the incisive foramen; distal tipping was measured at 10.9°, and no mesial movement was reported in the incisors. (Kircelli, Pektaş, & Kircelli, 2006) Escobar et al., however, reported 6 mm of distal movement and 11.3° of distal tipping at 7–8 months in 15 patients treated with a modified pendulum technique using two mini-screws; while 4.85 mm of distalization was observed in the second premolars, 0.5 mm of retrusion was noted in the upper incisors. (Escobar et al., 2007) Pendulum-type devices produce high distal tipping values due to the force applied by the TMA arm at the crown level; in the meta-analysis by Ceratti et al., an average distal tipping of 9.84° was reported for this group. (Ceratti et al., 2024)

In their systematic review encompassing 28 studies, Karvelas et al. highlighted a significant biomechanical distinction among palatal TAD-supported systems. While distal tipping in non-pendulum palatal TAD systems ranges from 0.01° to 11°, in pendulum + TAD combinations, this value rises to a significantly higher range of 7.34° to 22.8°. This finding indicates that the difference in tipping is largely attributable to the pendulum's lever mechanism (i.e., the application of force at the crown level). With regard to anchorage loss, it has been reported that no unwanted movement in the anterior segment was observed in any palatal TAD appliances other than the MGBM (Maino, Giannelly, Bernard, Mura) system. Additionally, it has been reported that the use of two miniscrews increases the amount of distalization and reduces distal tipping compared to a single miniscrew. (Karvelas et al., 2023)

The distal jet appliance has also found widespread use among systems modified with mini-screw support. Karaman et al. reported that a Class II molar relationship was successfully corrected in cases using a modified distal jet appliance supported by a palatal anchor screw; they noted that no unwanted movement was observed in the anchor tooth. (Karaman, Başçiftçi, & Polat, 2002) Cozzani et al. compared a skeletal-anchored distal jet (DS) appliance supported by two palatal mini-implants with a traditional tooth-supported distal jet (DJ) appliance; They reported that while the DS group achieved 4.7 mm of molar distalization and 2.8° of distal tipping, the traditional DJ group experienced anchorage loss in the form of 0.9 mm of first premolar mesialization alongside 4.4 mm of distalization. (Cozzani et al., 2014)

Keles et al. reported that in the Keles slider modification—which uses a palatal implant placed laterally to the midpalatal suture and posterior to the incisive foramen instead of the modified Nance button—they achieved 3 mm of parallel molar distalization over five months, with no loss of anchorage, incisor protrusion, or increase in overjet observed in the anterior segment. (Keles, Erverdi, & Sezen, 2003) Wilmes and Drescher, who introduced the Beneslider—a modification of this system—applied this appliance to 18 patients; achieving an average of 4.6 ± 1.5 mm of first molar distalization and $1.9 \pm 1.3^\circ$ of distal tipping after 6–10 months of treatment, and emphasized that transmitting the force close to the molar center of resistance enables body movement. (Wilmes & Drescher, 2010) In their study of 51 patients, Nienkemper et al. evaluated the relationship between age and the eruption of the second molars; they reported that a Class I molar relationship was achieved in all patients. They determined that the average mesial-distal displacement measured by the displacement of the trifurcation point was 3.6 ± 1.9 mm, and noted that no significant molar tipping or statistically significant movement in the anterior teeth was observed. (Nienkemper et al., 2014)

Kook et al. developed a modified palatal anchorage plate (MPAP) consisting of one mini-screw on one side of the midpalatal suture and two on the other, with the force vector adjustable via three notches on the arm. Total arch distalization, performed by applying approximately 300 g of force on each side, was completed in 20 adult patients in an average of 12.5 months; cephalometric analysis based on CBCT revealed 3.3 mm of distalization and 3.4° of distal tipping in the first molars, and significant molar intrusion was also reported. (Kook et al., 2014) Suzuki and Suzuki achieved a Class I molar relationship using the iPANDA (Indirect Palatal Miniscrew for Anchorage and Distalization Appliance) system, which relies on two miniscrews placed in the midpalatal suture; first molar distalization was measured at an average of 4.5 mm, and no significant changes were observed in tipping or the mandibular plane angle. (Suzuki & Suzuki, 2013)

Demir and Erdem have introduced a distalization system that combines a modified Nance appliance supported by a palatal mini-screw with a buccal mini-screw-lever arm combination. According to their findings, a force of 200 g was applied via the palatal mini-screw and 150 g via the buccal lever arm; a Class I molar relationship was achieved within five months, and body distalization was obtained. Additionally, it was reported that incisal movement was minimal. (Demir & Erdem, 2010)

AMDA (Advanced Molar Distalization Appliance), developed by Papadopoulos, is a mini-screw-supported system that applies force near the molar center of resistance using a tube-wire sliding mechanism anchored to two mini-screws placed in the anterior palatal region; it is designed to minimize anterior anchorage loss, molar tipping, and rotation. A key advantage of this appliance is that it can continue to be used as a skeletal anchorage unit in conjunction with fixed orthodontic treatment without requiring any modifications once distalization is complete. (Papadopoulos, 2010)

4. Clear Aligner Systems

Clear aligner systems are finding increasing application in the treatment of Class II malocclusion due to the advantages they offer in terms of aesthetics and patient comfort. With these systems, maxillary molar distalization is performed using a sequential protocol in which movement begins with the second molars and then proceeds to the first molars. When the second molar reaches approximately half of the target distance, distalization of the first molar begins; subsequently, the premolars and, in the final stage, the incisors are moved in sequence. This stepwise approach aims to prevent excessive stress on the intramaxillary anchorage. However, it remains a matter of debate in current studies whether the entire planned movement can be clinically achieved.

Studies evaluating the effectiveness of clear aligners in maxillary molar distalization reveal significant differences in the expected and actual rates of distalization. While Simon et al. reported a high accuracy rate of 87% in cases using attachments, other researchers have reported much lower values. (Simon, Keilig, Schwarze, Jung, & Bourauel, 2014) In their analysis of a three-dimensional digital model based on palatal rugae superimposition, Miao et al. obtained an average distalization of 1.25 mm and 1.41 mm in the first and second maxillary molars, respectively; they reported that these values corresponded to only 55.9% of the planned movement. (Miao et al., 2023) Mamani et al., however, found that the actual distalization was, on average, 1.32 mm less than planned for the first molar and 1.57 mm less for the second molar; they noted that the accuracy rates were 40.11% for the first molar and only 35.39% for the second molar. (Mamani, Sessirisombat, Hotokezaka, Yoshida, & Sirisoontorn, 2024) In their comprehensive evaluation covering the entire treatment process (including anterior tooth retraction), Li et al. reported success rates of 36.48% for the first

molar and 41.94% for the second molar; they emphasized that these rates were even lower in the group that underwent anterior retraction (31.50% and 35.63%). This finding highlights an important clinical reality. In this scenario, the distalization that appears to have been achieved with relatively high accuracy during the molar distalization phase can be largely reversed due to the reaction forces generated during the subsequent anterior retraction phase. (Li et al., 2023)

During distalization with clear aligners, distal tipping and buccal torque of the crown may occur at levels significantly higher than predicted in the virtual design. In their three-dimensional analysis, Miao et al. measured the average distal tipping difference as 5.36° and the buccal torque difference as 3.93° ; they reported that these deviations increased proportionally as the number of distalized molars increased. This trend is attributed to an insufficient torque resulting from the force being applied at the crown level rather than at the molar resistance center. (Miao et al., 2023) On the other hand, Mamani et al. did not find a statistically significant difference between the planned and actual angulation; however, they reported that while less distal tilt than planned was observed in the first molars, there was 1.98° more distal tilt than planned in the second molars. (Mamani et al., 2024) In the meta-analysis by Shen et al., the mean distal tipping values for the first and second molars were calculated to be 2.19° and 2.17° , respectively, based on radiographic evaluations. (Shen, Park, Chung, & Li, 2024)

Vertical dimension control remains one of the most controversial topics in distalization using clear aligners. In a systematic review and meta-analysis conducted by Park et al., an average of 0.26 mm of intrusion was observed for the maxillary first molar following a sequential distalization protocol; however, cephalometric analyses revealed no statistically significant changes in the SN-GoGn, SN-MP, and PP-GoGn angles. Researchers attribute this intrusion effect to the “turbo effect” created by the plaque completely covering the occlusal surfaces. (Park, Shen, Chung, & Li, 2024) On the other hand, it has been reported that high alveolar bone density and insufficient occlusal contact around the first molar in hypodivergent patients may negatively affect buccal torque control. Furthermore, regarding the effect of using attachments, while it has been reported that they increase the amount of distalization in the first molars, it has been emphasized that they have no significant effect on distal tipping and buccal torque in the second molars. (Miao et al., 2023)

Anterior anchorage loss also remains a significant clinical concern in distalization with clear aligners. In a study by Shen and colleagues, it was reported that a 0.39 mm protrusion—or loss of anchorage—occurred in the maxillary central incisors upon completion of the distalization phase; however, a 1.50 mm retraction at the incisal edge of the incisors was achieved by the end of the entire treatment. (Shen et al., 2024) In another study, it was reported that the retractor group was able to achieve retraction in only 56.10% of cases,

highlighting that even Class II elastics or mini-screws may not fully prevent loss of posterior anchorage. (Li et al., 2023)

When all these findings are considered together, it appears that clear aligner systems offer a valid treatment option in suitable cases requiring limited molar distalization (particularly in adult patients with high aesthetic expectations). However, according to findings in the current literature, clear aligner systems appear to have limitations compared to fixed orthodontic systems in terms of the degree of distalization, control of tipping, and anterior anchorage. When a large amount of distalization is planned, it is recommended to perform overcorrection or to incorporate a mini-screw for skeletal anchorage into the system in order to minimize the risk of insufficient expression.

RESULTS

Maxillary molar distalization plays an important role in orthodontic practice as it offers a conservative alternative to tooth extraction in the treatment of Class II malocclusion. An evaluation of the appliances studied reveals that intermaxillary and intramaxillary tooth-supported systems provide clinically acceptable distalization values; however, loss of anterior anchorage is a common limitation of these methods. With the introduction of temporary anchorage devices, this problem has been largely overcome; it has been demonstrated that frame-supported systems provide the most predictable results in terms of the degree of distalization and anchorage control. Clear aligner systems, on the other hand, are a preferred option, particularly among adult patients, due to their aesthetic advantages. When selecting an appliance, it is crucial to consider case-specific factors such as the required amount of distalization, patient compliance, and vertical growth patterns.

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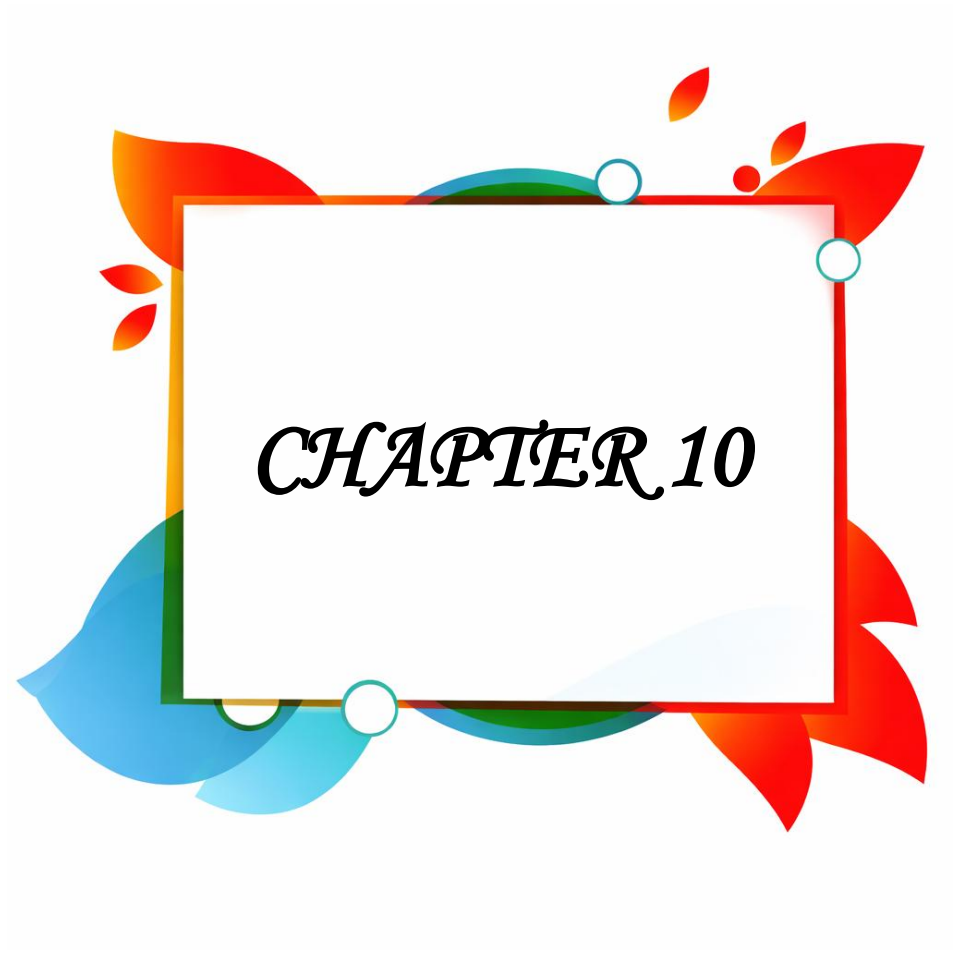
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Social Determinants of Periodontal Health: Implications for Health Equity and Clinical Practice

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1.Introduction

Periodontitis is an inflammatory disease characterized by the progressive destruction of the structures that support the teeth (Papapanou et al., 2018). Today, periodontal disease is considered not merely a local infection affecting oral health, but a global public health problem with significant impacts on an individual's quality of life, functional status, and overall health outcomes. The burden of periodontal disease is steadily increasing, particularly due to the aging population, the prevalence of chronic diseases, and disparities in access to healthcare services (Nascimento, Alves-Costa, & Romandini, 2024)(Figure 1).

Periodontitis is one of the most common chronic inflammatory diseases worldwide and is considered a significant public health problem. The disease has significant consequences on the oral health, quality of life and functional capacity of individuals due to its high prevalence and lifelong effects (Peres et al., 2019). Furthermore, periodontitis is a leading cause of tooth loss and places a significant economic burden on healthcare systems and societies. Recent global burden analyses show that periodontitis affects more than one billion individuals worldwide, and the disease burden continues to increase due to population growth and an aging population (Hashim et al., 2025).

The burden of periodontitis is not evenly distributed in the population. Epidemiological studies show that the prevalence and severity of the disease are significantly influenced by both biological factors (age, gender, genetic predisposition) and socioeconomic factors (income level, education, living conditions, access to healthcare) (Albandar, 2025). Higher prevalence of periodontitis, more advanced clinical destruction, and greater tooth loss are reported, particularly in individuals with low socioeconomic status. These findings suggest that periodontal diseases cannot be explained solely by

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individual behaviors or biological risk factors, but must be evaluated within a broader social context (Borrell & Crawford, 2012).

Social determinants refer to the effects of the living and working conditions of individuals on their health. Current evidence shows that factors such as income level, education, access to healthcare, and health literacy are associated with the risk of periodontal disease and treatment outcomes. Furthermore, it is reported that social disadvantage can affect periodontal health through behavioral and biological mechanisms (Li, Zhang, Wu, & Song, 2025).

Therefore, understanding periodontal health inequalities requires looking beyond traditional biological risk factors and considering the social determinants that influence disease burden. This chapter reviews the social determinants of periodontal health and discusses their impact on periodontal disease development and access to periodontal care. It also highlights the clinical implications of these factors and their role in promoting periodontal health equity.

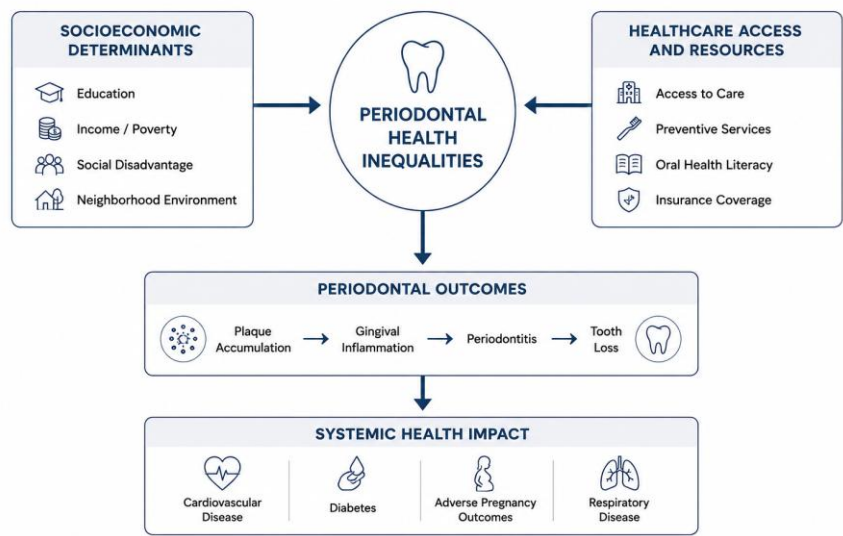


Figure 1. Conceptual framework illustrating the relationships among socioeconomic determinants, healthcare access, periodontal health inequalities, periodontal disease outcomes, and systemic health impacts.

2. Social Determinants of Periodontal Health

Periodontal health is influenced by a complex interaction of biological, behavioral, environmental, and social factors. While dental biofilm is recognized as the primary etiological factor in periodontitis, substantial variations in disease prevalence and severity cannot be explained by microbial factors alone. Increasing evidence suggests that social determinants, including socioeconomic status, education, health literacy, living conditions, and access to healthcare, play an important role in shaping periodontal health outcomes. Individuals exposed to

social disadvantage are more likely to experience a higher burden of periodontal disease and poorer treatment outcomes than their more advantaged counterparts (Li et al., 2025).

2.1. Socioeconomic Status

Socioeconomic status is a key determinant of periodontal health. Individuals with lower income and educational attainment are more likely to develop periodontitis and experience poorer periodontal outcomes. These inequalities are associated with differences in health literacy, access to preventive and therapeutic dental services, and exposure to adverse living and working conditions. Furthermore, recent evidence suggests that the cumulative burden of social disadvantage may contribute to periodontal disease risk independently of traditional behavioral risk factors (Liang et al., 2024).

2.1.1. Income

Income influences periodontal health through its impact on living conditions, access to healthcare, and the ability to obtain preventive and therapeutic dental services. Individuals with lower income levels are more likely to experience severe periodontitis, greater attachment loss, and increased tooth loss. Evidence also suggests that the relationship between income and periodontal health persists even after adjustment for behavioral risk factors, indicating that financial disadvantage may independently contribute to periodontal disease risk (Borrell, Beck, & Heiss, 2006; Celeste, Oliveira, & Junges, 2019).

2.1.2. Education

Education is an important determinant of periodontal health and is closely linked to health literacy and health-related behaviors. Individuals with higher educational attainment are generally more likely to adopt preventive oral health practices and utilize dental services regularly. Recent evidence suggests that higher levels of education are associated with a lower risk of periodontal disease, highlighting the role of educational attainment in shaping periodontal health outcomes (Warren, Himmelstern, Muller, Grodsky, & Demmer, 2025).

2.1.3. Occupation

Occupational status may influence periodontal health through differences in income, working conditions, psychosocial stress, and access to healthcare. Individuals employed in lower-skilled occupations often experience greater exposure to adverse working environments and health-related risk factors. Occupational demands may also limit opportunities for regular dental visits and engagement in preventive oral health behaviors. In addition, workplace-related stress has been associated with behavioral and physiological responses that may adversely affect periodontal health (Schuch, Peres, Singh, Peres, & Do, 2017). Certain occupational conditions, particularly shift work, may contribute to an

increased risk of periodontal disease through mechanisms involving psychological stress, disrupted circadian regulation, and unhealthy lifestyle behaviors (Jung et al., 2023).

2.2. Health Literacy and Oral Health Literacy

Health literacy refers to an individual's ability to obtain, understand, evaluate, and use health information to make appropriate health-related decisions. In the context of oral health, oral health literacy plays an important role in shaping preventive behaviors, treatment-seeking patterns, and the utilization of dental services (Baskaradoss, 2018). Individuals with low levels of knowledge about oral health are less likely to practice effective oral hygiene, attend regular dental visits, and recognize early signs of oral disease (Firmino et al., 2018). Consequently, limited oral health literacy can lead to late diagnosis and worse oral health outcomes.

2.3. Environmental and Community Factors

Environmental and community factors can substantially influence periodontal health. The conditions in which individuals live, work, and interact with their communities may affect both the risk of periodontal disease and access to oral healthcare services. Individuals living in socioeconomically disadvantaged neighborhoods often face barriers to preventive dental care and may have limited access to oral health resources (Obeidat, Heaton, Tranby, O'Malley, & Timothé, 2024). Geographic disparities, particularly between urban and rural areas, can further contribute to inequalities in oral health outcomes by affecting the availability and utilization of dental services (Zare, Bahrami, Bastani, & Kavosi, 2024). Community-level factors such as transportation, social support, and neighborhood resources may also influence healthcare-seeking behaviors and access to care (Obeidat et al., 2024). In addition, adverse environmental conditions may increase chronic stress and reduce opportunities for maintaining healthy lifestyles, both of which can negatively affect oral health (Petersen & Kwan, 2011). These findings suggest that periodontal health is shaped not only by individual characteristics but also by the broader social and environmental context in which people live. Racial and ethnic disparities in periodontal health add a further dimension to this picture. Evidence consistently shows that racial and ethnic minority populations experience a higher burden of periodontitis even after controlling for income and education, suggesting that structural racism and differential exposure to social disadvantage contribute independently to oral health inequalities (Borrell & Crawford, 2012).

3. Pathways Linking Social Determinants to Periodontal Disease

3.1 Behavioral Pathways

Behavioral factors represent an important pathway through which social determinants influence periodontal health. Individuals experiencing

socioeconomic disadvantage are more likely to be exposed to behavioral risk factors associated with periodontal disease, including inadequate oral hygiene practices, tobacco use, unhealthy dietary patterns, and irregular dental attendance (Li et al., 2025). Poor adherence to oral self-care practices has been associated with increased biofilm accumulation and poorer periodontal health outcomes (Schüz, Sniehotta, Wiedemann, & Seemann, 2006).

3.2 Psychosocial and Biological Pathways

In addition to influencing health-related behaviors, social determinants may affect periodontal health through psychosocial and biological mechanisms. Individuals exposed to socioeconomic disadvantage often experience higher levels of chronic stress, financial insecurity, and adverse life circumstances. Persistent exposure to these stressors may alter neuroendocrine and immune responses, thereby increasing susceptibility to inflammatory diseases, including periodontitis (Macri et al., 2024). Chronic stress has been associated with dysregulation of the hypothalamic–pituitary–adrenal (HPA) axis and increased secretion of stress-related hormones such as cortisol, which may influence host immune responses and periodontal tissue health (Hensel, Nicholson, Anderson, & Goma, 2024). Furthermore, elevated stress levels and salivary cortisol concentrations have been linked to greater periodontal disease severity, suggesting that psychosocial stress may contribute to periodontal disease progression through biological pathways (Hingorjo, Owais, Siddiqui, Nazar, & Ali, 2025).

3.3 Impact on Periodontal Inflammation

Social disadvantage may contribute to periodontal disease progression by influencing inflammatory and immune responses. Chronic exposure to psychosocial stress and adverse living conditions has been associated with alterations in immune regulation and increased systemic inflammatory burden (Hensel et al., 2024). These changes may affect the host response to dental biofilm and promote a more destructive inflammatory environment within periodontal tissues (Macri et al., 2024). Elevated levels of pro-inflammatory mediators, including interleukin (IL)-1 β , IL-6, and tumor necrosis factor (TNF)- α , have been implicated in periodontal tissue destruction and disease progression (Neurath & Kesting, 2024).

4. Periodontal Health Inequities and Access to Care

Access to periodontal care is an important component of oral health equity. Despite advances in preventive and therapeutic periodontal interventions, access to these services remains uneven across populations. Financial barriers, lack of dental insurance, geographic limitations, and workforce shortages may restrict access to periodontal care, particularly among disadvantaged communities (Watt et al., 2019). In numerous contexts, those with the highest treatment needs are

frequently the least likely to access timely and adequate periodontal care, thereby perpetuating inequalities in oral health outcomes (Peres et al., 2019).

Limited access to periodontal services may result in delayed diagnosis, progression of periodontal disease, and increased tooth loss. In addition, inequalities in access may affect participation in supportive periodontal care programs, which are essential for maintaining long-term periodontal stability. Recent evidence suggests that reducing barriers to oral healthcare and improving the availability of preventive services may contribute to more equitable periodontal health outcomes at the population level (Watt, 2023).

5. Implications for Clinical Practice

Recognition of social determinants of health has important implications for periodontal practice. Although periodontal diagnosis and treatment planning are traditionally based on clinical findings, broader social and environmental factors may also influence treatment adherence, maintenance attendance, and long-term outcomes (*Global Oral Health Status Report*, 2022). Considering these factors may help clinicians better identify patients who are at increased risk of disease progression and challenges in maintaining periodontal health.

Patient-centered care has become an essential component of contemporary healthcare. Effective communication, individualized oral health education, and shared decision-making may improve patient engagement and support adherence to periodontal treatment recommendations. Such approaches may be particularly important for individuals facing social and economic barriers that limit access to oral healthcare services (Harnagea et al., 2018).

Incorporating preventive and risk-based approaches into routine periodontal care has the potential to reduce oral health inequalities. Early identification of at-risk individuals, broader access to preventive services, and consistent long-term supportive care are all critical steps in lowering the burden of periodontal disease at both the individual and population level. Reaching these goals, however, requires coordinated effort among dental professionals, public health practitioners, and policymakers to build more equitable oral healthcare systems (*Global Oral Health Status Report*, 2022; Righolt, Jevdjevic, Marcenes, & Listl, 2018).

6. Future Directions

Periodontal health inequalities are closely linked to wider social and structural determinants, and reducing these disparities will require long-term and coordinated action across research, policy, and clinical practice. Future studies should prioritize longitudinal approaches to improve understanding of how social disadvantage accumulates over time and influences periodontal health outcomes throughout the life course (Albandar, 2025; Li et al., 2025). Most existing evidence is derived from cross-sectional studies, which limits causal inference.

Prospective cohort studies that integrate both clinical periodontal measures and comprehensive social determinant data — including neighborhood-level indicators, occupational history, and chronic stress exposure — would substantially advance understanding of the mechanisms through which social factors drive disease burden (Tsakos, Watt, & Guarnizo-Herreño, 2023). Furthermore, greater use of intersectional analytical frameworks is needed to examine how overlapping dimensions of disadvantage, such as income, race, gender, and geography, jointly influence periodontal health outcomes (Liang et al., 2024).

Digital health technologies represent a promising avenue for reducing disparities in access to periodontal care. Teledentistry and artificial intelligence-supported diagnostic tools have the potential to extend periodontal screening and preventive services to geographically isolated and socioeconomically disadvantaged populations (Drafta, Macris, & Petre, 2025). Emerging evidence suggests that teledentistry platforms supported by AI-based risk evaluation may improve the timely identification of periodontal disease and facilitate more efficient patient triage in underserved communities (Farina et al., 2025). Mobile health applications designed to improve oral health literacy and self-care adherence may also support patients with limited access to regular dental services. However, realizing this potential requires addressing structural barriers such as digital literacy gaps, internet access inequalities, and the absence of reimbursement frameworks for remote oral health services (Kengne Talla et al., 2025). Future research should evaluate the effectiveness and equity impact of these digital interventions across diverse population groups.

At the policy level, reducing periodontal health inequalities will require moving beyond clinical interventions toward structural change. The WHO Global Strategy and Action Plan on Oral Health 2023–2030 calls for the integration of oral health into universal health coverage frameworks and primary health care systems, with an explicit emphasis on health equity (*Global Oral Health Status Report*, 2022). The "health in all policies" approach, which embeds oral health considerations into broader social, educational, and economic policy domains, offers a viable pathway for addressing the upstream determinants of periodontal disease (Serban et al., 2025; Tsakos et al., 2023). Expanding community-based periodontal programs, reforming dental insurance systems to cover preventive services, and training oral health professionals in social determinants-informed care are among the concrete steps that can translate this agenda into practice. Collaborative efforts between periodontal researchers, public health practitioners, and policymakers will be essential to build the evidence base and the political will needed to achieve more equitable periodontal health outcomes at the population level (Albandar, 2025).

7. Conclusion

Periodontal disease is not simply a product of individual oral hygiene habits. The evidence reviewed in this chapter shows that socioeconomic status, education, income, occupation, housing conditions, psychosocial stress, and access to care all shape periodontal health in meaningful ways. These factors rarely act in isolation; they accumulate over the life course and interact with behavioral and biological pathways to produce the disparities observed across populations (Albandar, 2025; Liang et al., 2024). Addressing periodontal disease therefore requires looking beyond the oral cavity and recognizing the broader structural conditions that make some groups consistently more vulnerable than others.

For clinical practice, this means incorporating social determinants into risk assessment and treatment planning. For public health, it means designing interventions that target upstream factors rather than individual behavior alone. The WHO Global Strategy and Action Plan on Oral Health 2023–2030 provides a useful framework, but translating its goals into equitable outcomes will depend on intersectoral collaboration, sustained policy commitment, and research that moves beyond cross-sectional designs toward longitudinal, population-based evidence (*Global Oral Health Status Report*, 2022; Tsakos et al., 2023). Periodontal health equity can only be achieved when the social determinants of disease are addressed with the same urgency and commitment as its biological mechanisms.

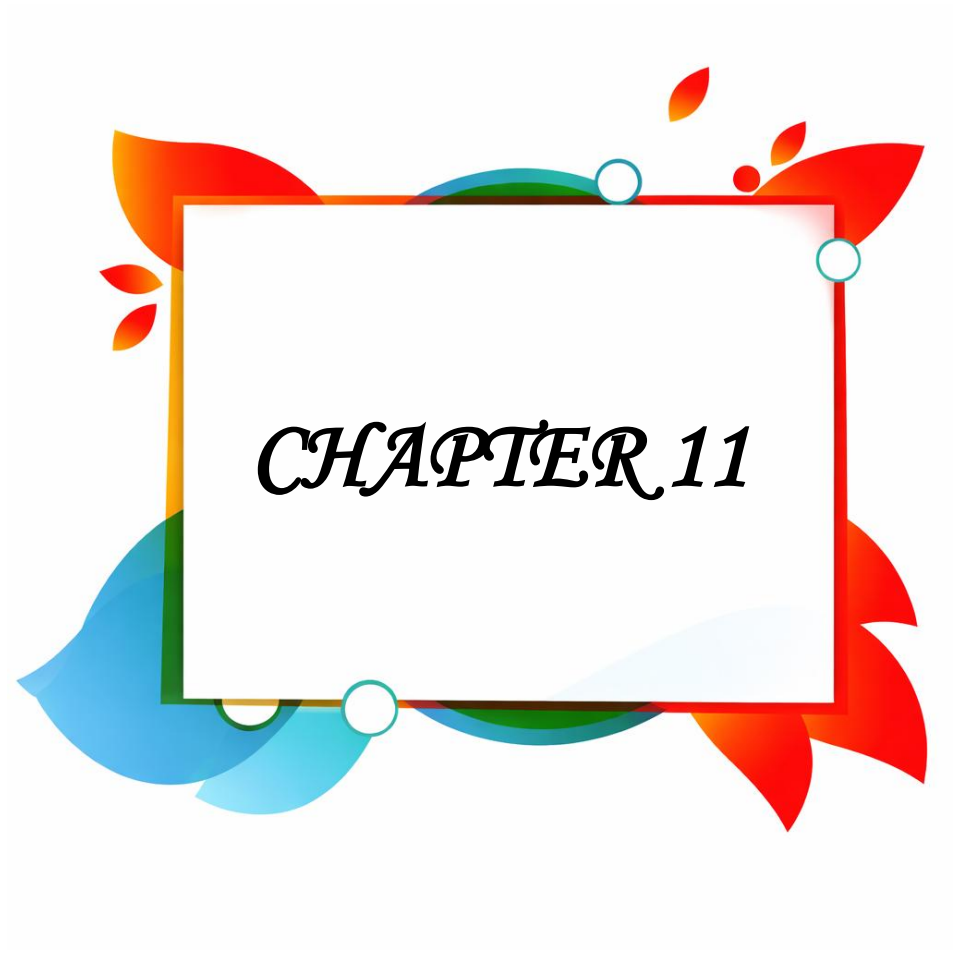
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Shaping The Future of Pathology: Use of Artificial Intelligence as a Component of Telepathology and Digital Pathology

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INTRODUCTION

Digital pathology (DP) is a term used for transformation of conventional light microscope images into digital images. The term “DP” evolved to involve several modalities of imaging technologies such as transmission of static images, robotic static/dynamic telemicroscopy, real-time video telemicroscopy since its emergence in 1990s. The most used form of DP is whole-slide imaging (WSI).¹ Whole slide imaging, introduced to clinical use in 1999, allows converting tissues on glass slide into high-resolution virtual slide in a digital platform.²

With development of innovations in DP; pathology diagnosis workflow has changed like it has never been before. Besides telepathology became easier to perform, generation of large amount of pathological data and combining DP with artificial intelligence (AI) is possible. At this point we have reached, AI algorithms (machine learning and deep learning) are used for the detection, segmentation, registration, processing, and classification of digitized pathological images.³ Recently, the combination of DP and AI technologies has changed the point of view of pathologists on their daily work. However, integration of these systems to daily routine has remained limited despite the fact that scientific community makes pressure for refined and reproducible diagnoses. This limitation is justifiable since transition of conventional pathology practices into DP requires organizational, functional and technical infrastructure.⁴

In the beginning, DP was used for transforming WSI into digital platform. For this purpose, advanced slide scanning technologies were developed. Instead of examining tissue glass slides through a microscope, DP makes it possible for a pathologist to examine digital images on a computer monitor. Recently, however, this term evolved to a generic term that includes AI-based approaches for detection, segmentation, diagnosis, and analysis of digitalized images.³ As an

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application of telepathology in DP, DP slides may be transmitted through internet and evaluated by an expert in a distant location.⁵ Through internet networks, DP enables real time consultation on a case with many experts in pathology at different facilities, and also, in different countries.³ Pathologists using DP properly may combine traditional handcrafted feature approaches with deep learning methodologies involved in AI. Data fusion algorithms for combining radiologic, histologic, and molecular measurements has potential to improve disease characterization.⁶ As pathology diagnosis depends on detection of patterns, AI is convenient for pathology practice.⁷ AI is a branch of computer science that enables algorithm development for tasks that require problem solving, decision-making, visual perception, and pattern recognition which are mainly thought to be components of human intelligence. Pattern analysis of tissues of patients with cancer risk is one of the areas of interest of AI. AI may revolutionize pathology in terms of faster and more accurate diagnoses, better treatment decisions, and improved patient outcomes.⁸

As mentioned above, digital applications that depend on machine learning may help pathologists in many aspects. Use of AI in pathology may also reduce human and infrastructural resource burden by creating online data repositories that can be used for diagnosis of pathological specimens.⁷ Hence, pathology departments are rapidly being digitalized and taking advantage of transforming diagnostic modes and workflow from microscopic diagnosis to digital image computer review. There are also good examples of fully digitalized pathology departments in the World. On the other hand, there are some challenges in implementation of this technology to pathology departments. For instance; the quality stability, cost of the scanner, the validation of the system, the reengineering of the workflow and perspective of pathologists for the DP that needs to change. Despite its high potential, another challenge for AI is that it is still limited to boundaries of auxillary diagnostics field. With the spread of DP, formation of pathological procedures will change.⁹

MATERIALS AND METHODS

This narrative review was conducted by enetring the keywords “artificial intelligence”, “digital pathology” and “telepathology” to the Pubmed Database. In this field, we obtained a total of 81 results. Since artificial intelligence studies has a relative short history and AI articles do not go back more than five years, articles within last five years were included. Hence, a total of 40 articles were included into the study. Articles in English with full-text or with an explanatory abstract were included into the study. Then, articles were reviewed by two authors for relevance to the subject. Then, remaining 34 articles were investigated in details. Of these 34 articles, 15 were original articles, 16 were reviews and 3 were editorials.

IMPLEMENTATION OF AI IN DIGITAL PATHOLOGY

Digital pathology has changed the understanding of primary diagnosis in pathology practice. Digital pathology supports remote diagnostic reporting with flexible working hours and enables delivery of service and expert report availability even in hard times such as pandemics. This fact has also gained importance with the idea of combining DP with AI. Thus, AI-based algorithms became more frequently used in clinical practice.¹⁰ Besides the difficulties, the COVID-19 pandemic has accelerated use of telemedicine and telehealth in many facilities and schools.¹¹ The combination of DP with AI has changed pathology practice in many perspectives such as diagnostic applications, prognostic and predictive applications, integration with patient genomic and genetic profiles and pathology workflow efficiency. AI is also utilized in pathology training and education.¹⁰ Despite the fact that it is limited to a few pathology laboratories, DP along with WSI can be applied to AI-based algorithms which improves diagnostics accuracy, planning personal treatment methods and clinicians' decision making.⁴

With use of AI, morphological patterns can be easily and quickly recognized and diagnosis/treatment can be guided in various locations using the fundamental process of slide screening.¹² However, despite the advances in this field in recent years, DP is not available in many laboratories and it usually focuses on research purposes. Nevertheless, there is a progressive increase in number of institutions utilizing DP. AI provides advantage to pathologists in historically problematic issues such as shortening decision-making time increasing the standardization of reports.⁴

Success of AI-based systems mainly relies on clinical relevance of the input data. Analysis of single data may differ from combined data.¹⁰ In their report, Eccher et al. stated that AI-based deep learning tools has provided successful outcomes in prostate biopsy screening for cancer, computer-assisted PD-L1 scoring in lung cancer specimens and automatic evaluation of breast cancer IHC biomarkers (estrogen receptors, progesterone receptors, and Ki-67 proliferative index).⁴ Beside assessment of proliferation (Ki-67) index, AI may also be used in cytonuclear pleomorphism/degree of atypia cellularity and counting mitotic figures, scoring of tumour infiltrating lymphocytes assessment.¹⁰

Importance of AI and telepathology has been revealed particularly in COVID-19 pandemic. The pandemic caused delays in detection in cancer diagnosis and treatment. In these circumstances, AI and telepathology emerged as promising tools to provide timely and accurate diagnosis. Regardless of techniques used in slides (hematoxylin and eosin (H&E) or immunohistochemistry), pathological samples can be analyzed, interpreted and accessed remotely by the help of telepathology. Also, scanned images can be sent as slides to pathologists anywhere in the World in order to make accurate and rapid evaluation.

Telepathology and AI can also be useful in monitorization and follow-up of patients with cancer.¹³ Telepathology may be addressed in three categories: static (pre-captured images), dynamic (live images), and virtual slide systems (a combination of previous categories using WSI) according to operational and diagnostic requirement.¹⁴ Microscope-dedicated optic cameras and recently smartphones are used by pathologists for creation of static images.¹⁵ As the technology of sample preparation and hardware devices improve, DP has been applied for diagnosis and evaluation of various diseases. Hence, WSI which allows the transfer of the whole tissue section or cell sample on a glass slide into a high-resolution digital image has been widely used.¹⁶ WSI utilizes virtual microscopy and has positive impact on pathology workflow, reproducibility, dissemination of educational material, expansion of service to underprivileged areas, and innovative movement by enabling institutional collaboration. This procedure is also approved by US Food and Drug Administration.¹⁷ Telepathology along with AI, machine learning and deep learning may give more information from WSI images and thus, enhance the capability of pathologists to interact each other through an image bank for diagnostic processes and prognosis.¹⁸ Speaking of image banks, Chen et al. investigated the performance of deep learning and DP for storage capacity and they reported that DP images could be effectively compressed before transmission to DL-based telepathology applications.⁵

Basically, pathological slides are stained with H&E. AI models give rise to use of WSI of H&E-stained images as diagnostic tools.³ Success of this procedure mainly depends on two factors: technical factors for slide preparation and the color tone differences between H&E stain and WSI in laboratories. In a multi-centered study in which the impact of these differences on diagnosis was evaluated, Abe et al. developed an AI-based system for the pathological diagnosis of gastric biopsies. WSIs of 4511 gastric biopsy tissues from 984 patients were evaluated with AI and the concordance rate for AI was found to be satisfactory. The failure points of AI were linked to differences in the color of H&E staining.¹⁹

Another good example for AI use in pathology is SMILE (Secretary-Mimicking Artificial Intelligence) developed by Jay et al. Considering the fact that secretarial tasks of translating these interpretations into final pathology reports frequently performed by pathologists, a programme aimed to type and finalize pathology reports was developed. This AI programme enabled for the pathologists to save time and focus on on slide interpretation. Thus, workload and stress of pathologists was reduced, and their productivity was increased.²⁰ In another study, AI was used for teleconsultation, teaching platforms, fluorescence, and cytology. For this purpose the DigiPatICS Project was carried out in network of 8 hospitals involving 168 pathologists and over 1 million slides each year. It was determined that the system provided an effective and safe diagnostic tool with clear benefits for diagnosis quality and patient safety.²¹ In another project

by Browne et al., Denoising Diffusion Probabilistic Model was developed to predict super-resolution pathology slide images from low-resolution inputs. From the dataset, a total of 150 000 images were chosen randomly. They concluded that the system could decrease the reliance on costly slide scanners for acquiring high-quality pathology slide images, while also streamlining clinical workflow and expanding the scope of ophthalmic telepathology.²² Computer-based systems assist pathologists on histopathological patterns, molecular, and clinical information. In Colombia, study protocol of the GLORIA digital pathology network was presented to contribute to diagnosis of complex malignant tumors. It helped to expand oncopathology service across all Colombian regions by making a freely accessible national histopathological image database possible.²³

In a study by Movahed-Ezazi et al., used Stimulated Raman histology (SRH) which allows microscopic examination of fresh tissue intraoperatively via an ex vivo imaging modality. It enables remote telepathology review and gain time and labor. The method eliminates tissue loss and artifacts, and enhances the accuracy of diagnosis. The researchers used surgical specimens of 47 subjects with SRH images and WSIs stained with H&E. When diagnostic concordance of SRH and WSI diagnoses were investigated, it was found that SRH could be implemented as a rapid intraoperative diagnosis method along with other conventional methods.²⁴

Liotta et al. investigated the effectiveness of a combination of AI and Laser Capture Microdissection (LCM) which is a laser technology used to isolate or capture specific cells in a complex heterogeneous tissue section. They concluded that emerging LCM technology is combining digital and AI guided remote imaging with automation, and telepathology, to achieve multi-omic profiling that was not previously possible.²⁵

ResNet50, a deep convolutional neural network, was used in another study in order to determine association of gastritis with gastric cancer. The Operative Link on Gastric Intestinal Metaplasia Assessment was different in only 438 images (7.6%) between pathologists and the AI system. Thus, ResNet50 successfully identified minimal IM areas that pathologists missed during the review.²⁶ In another study by Lee et al. programmed death-ligand 1 (PD-L1) in urothelial carcinoma was analysed with AI. In the guidance of AI, the complete agreement was raised to 93.9%. AI was found to be helpful in eliminating discrepancies between pathologists.²⁷

Despite the fact that number of fully-digital laboratories is inadequate, experts on DP from different countries gather in many occasions to make collaboration and share their experiences with each other. For instance, “Digital Pathology All Stars” event was conceived by the University and Hospital Trust of Verona in 2022 and experts had a chance to come together.²⁸

The literature researches on DP and its above-mentioned components are also useful to raise awareness in this field. In a study on diagnostic value of DP was investigated, Azam et al., in their systematic review and meta-analysis, utilized twenty-five studies which examined a total of 10 410 histology samples. the agreement percentage was 98.3% across 24 studies. A total of 546 major discordances, mostly related to assessment of nuclear atypia, grading of dysplasia and malignancy, were reported across 25 studies. The performance of DP was equal to light microscopy.²⁹ In another review on oral squamous cell carcinoma with 1798 articles in 2023, teleradiology was reported in nine studies and both telepathology and DP were mentioned in seven studies.³⁰ It is inevitable that as DP spreads to more laboratories, the literature on DP will also expand in concordance.

CHALLENGES IN COMBINING DIGITAL PATHOLOGY AND AI

AI has been successfully applied to radiology and cardiology. This was an inspiration for pathologists but there are also challenges in integration of DP to pathology practice. Firstly images in pathology has a generation process (tissue biopsy, specimen preparation, and staining before digitization) unlike these disciplines. It is more difficult to develop AI algorithms capable to conserve gain in productivity from automation in pathology. Additionally, cardiology and radiology has the advantage to create 3 and 4 dimensional images which increases the diagnostic accuracy. On the other hand, pathologic images normally are digitized for 2-dimensional presentation.³¹ In order to implement AI into pathology, digitalization is needed. Even though number of digitalized laboratories is increasing, the process needs funding. Particularly in low-income countries, funding required for WSI scanners, associated information technology infrastructure, and the storage requirements needed for the large sizes of whole-slide images still remains as a challenge. Digital workflow requires technology infrastructure such as a broadband internet connection, a sample tracking system integrated with the laboratory information system and a WSI scanner, and pathologists' workstations. Quality control and validation tests takes a significant time. Molecular pathology and genomic research databases should be combined in order to use DP properly and enable sharing cases and perform teleconsultation.³² Technically, color variations of tissues challenges computational interpretation of digital slide images. These variations may rise from slide preparation, staining, and even scanners used for WSIs. Algorithms developed to make a decision should be familiar to these variations. As mentioned above, another technical challenge is that most of the scanners are capable of creating only 2-dimensional images. On the other hand, microscopes give pathologists opportunity to get advantage from the "depth" dimension. Especially, this "depth" helps pathologists when investigating mitotic activity. The availability of depth (or z-axis) in the near future will be useful in developing more intelligent and efficient approaches for detecting, segmenting, analyzing,

and interrogating 3D stacks of digitized slide images.⁶ Problems about the device expense and algorithms can be overcome as long as AI developers and pathologists work together and empathize each other for challenging issues.⁸ AI system required for a laboratory should be investigated in details and purchased based on clinical application, specific needs and budget of the laboratory, as well as the personal preference of the telepathologists involved. There is an ongoing need for subspecialist consultation, improving diagnostic accuracy and workflow.³³

CONCLUSION

AI technology is inevitable for pathologists with its feasibility and power. AI will certainly transform the pathology practice. It will take its place in every diagnostic process in the near future.³⁴ However, our review revealed that DP has many challenges today. Scarce of digitalized laboratories, traditional 2-dimensional nature of pathology images, funding-dependent processes and lack of information technology infrastructure are just a few examples of the many problems. On the other hand, digitalization of pathology and combination of this system with AI has a potential to transform pathology practice since it provides advantages that cannot be ignored.

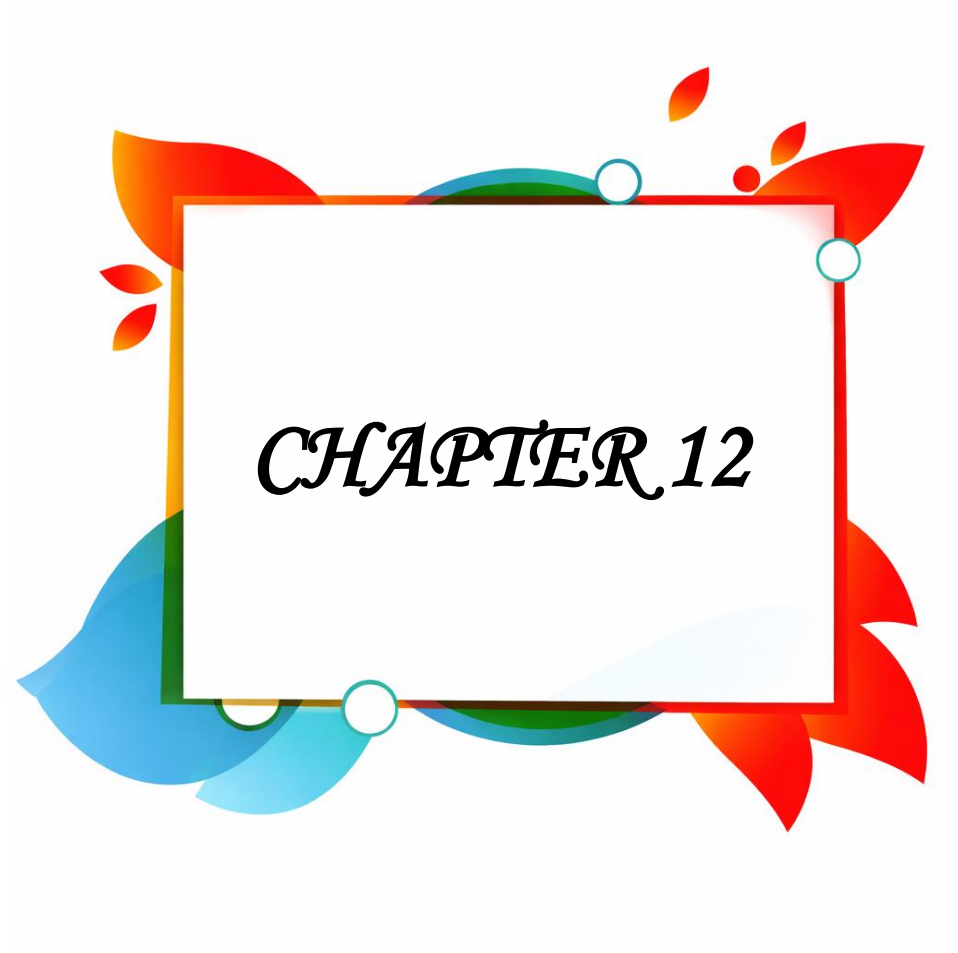
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Micrornas in the Pathogenesis of Sepsis-Associated Disseminated Intravascular Coagulation

Gülfem Erbil¹

1. INTRODUCTION

Disseminated intravascular coagulation (DIC) is a multifactorial coagulopathic disorder with high mortality, defined by the concurrent presence of widespread intravascular thrombosis and a bleeding tendency, particularly under conditions of sepsis and critical illness (Elawad et al., 2021). The occurrence of DIC among patients with sepsis can reach up to 30–50%, and the marked increase in mortality among patients who develop DIC underscores its clinical significance. However, in routine clinical practice, the diagnosis of DIC is often established at a late stage, after substantial deterioration in coagulation parameters has already occurred; this substantially limits opportunities for early diagnosis and targeted intervention (Caserta et al., 2016). Current diagnostic approaches fail to adequately reflect the underlying molecular mechanisms of DIC and lack specific biomarkers capable of predicting its early onset.

The development of DIC reflects multiple interacting pathways, including activation of inflammation, excessive stimulation of the coagulation cascade, endothelial dysfunction, and platelet consumption. The incomplete elucidation of this complex biological network at the molecular level hampers the development of early DIC specific biomarkers and novel therapeutic targets.

MicroRNAs (miRNAs) are small non-coding RNAs involved in post-transcriptional gene regulation and function as essential modulators of a wide range of biological processes such as inflammation, coagulation, and vascular homeostasis. Although several miRNAs have been linked to sepsis and coagulation disorders, systematic and discovery-based studies focusing on miRNAs directly linked to DIC pathogenesis particularly those not previously identified remain extremely limited. This represents a major gap in the current understanding of the molecular mechanisms underlying DIC (Formosa et al., 2022).

In light of these limitations, there is a pressing need for integrative and mechanism-oriented approaches to better elucidate the molecular landscape of

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DIC, particularly in the context of sepsis. Identifying both known and previously uncharacterized (novel) miRNAs involved in DIC may provide critical insights into the regulatory networks underlying coagulation abnormalities, inflammatory responses, platelet consumption, and endothelial dysfunction. A comprehensive evaluation of these miRNAs at both the molecular and clinical levels may not only enhance our understanding of DIC pathophysiology but also facilitate the identification of novel biomarker candidates with potential utility in early diagnosis and risk stratification. Such advances could ultimately lay the groundwork for the advancement of more improved diagnostic tools and mechanism-based therapies.

2. DIC PATHOGENESIS

DIC is a severe, potentially fatal hemostatic disorder marked by the aberrant activation of systemic coagulation, leading to widespread microthrombus formation concomitant with a bleeding tendency (Iba et al., 2023). DIC is not a primary disease; rather, it is defined as a secondary syndrome that arises in the course of various clinical conditions, including sepsis, severe infections, trauma, malignancies, and obstetric complications. Clinically, DIC is characterized by the coexistence of organ dysfunction associated with microvascular thrombosis and hemorrhagic complications due to depletion of coagulation factors and platelets. Although conventional coagulation parameters such as prothrombin time, platelet count, fibrinogen levels, and fibrin degradation fragments are used in the diagnostic process, these markers typically become apparent only in the later phase of disease progression (Levi et al., 2018).

The molecular pathogenesis of DIC is based on complex and bidirectional interactions between the inflammatory and coagulation systems. Particularly during sepsis, the activation of immune cells via toll like receptors by pathogen associated molecular patterns (PAMPs) and damage associated molecular patterns (DAMPs) causes an increased release of proinflammatory cytokines, including tumor necrosis factor alpha (TNF- α), interleukin 6 (IL-6), and interleukin 1 beta (IL-1 β). Monocytes and endothelial cells upregulate tissue factor expression in response to these cytokines, resulting in widespread activation of the extrinsic coagulation cascade. Consequently, thrombin generation is enhanced, and fibrin formation is accelerated at the systemic level (Liaw et al., 2016; Gong et al., 2025).

Increased thrombin generation not only promotes fibrin clot formation but also triggers activation of platelets and aggregation, thereby contributing to the development of microvascular thrombosis (Gando et al., 2025). Concurrently, the suppression of physiological anticoagulant mechanisms, namely antithrombin, protein C, and protein S systems, leads to a loss of control over coagulation activation. Sepsis associated endothelial dysfunction results in reduced expression of endothelial protein C receptor and thrombomodulin, thereby

weakening anticoagulant and anti inflammatory signaling pathways. In addition, suppression of the fibrinolytic system, particularly through elevated extent of plasminogen activator inhibitor 1, contributes to the persistence of fibrin accumulation and the progression of microcirculatory impairment (Popescu et al., 2022; Yang et al., 2025).

In the later stages of DIC, widespread thrombin generation and fibrin formation lead to the rapid consumption of platelets and coagulation factors, resulting in a pronounced bleeding tendency that accompanies ongoing microthrombosis. This dual pathophysiology, the simultaneous presence of thrombosis and bleeding, represents a defining feature that distinguishes DIC from other coagulopathies. Current evidence indicates that DIC arises not only from excessive activation of the coagulation system but also as an integrated consequence of inflammation, endothelial dysfunction, and cellular responses. Nevertheless, the regulatory mechanisms underlying this complex molecular network remain incompletely understood, and the role of post transcriptional regulators, in particular, has emerged as a critical area of investigation.

3. THE RELATIONSHIP BETWEEN SEPSIS AND DIC

Sepsis is a systemic inflammatory syndrome associated with a disrupted host response leading to failure of multiple organ systems and represents the most common and potent clinical trigger for the development of DIC. During sepsis, the innate immune response activated by microorganisms or their toxins disrupts the physiological balance between the inflammatory and coagulation systems (Takeshita et al., 2023). Excessive release of proinflammatory cytokines enhances tissue factor expression in endothelial cells and monocytes, thereby accelerating thrombin generation and promoting widespread fibrin formation at the systemic level. Concurrently, sepsis associated endothelial injury is characterized by increased vascular permeability, upregulation of adhesion molecules, and loss of anticoagulant surface properties; these alterations facilitate the development of microvascular thrombosis and further exacerbate DIC pathogenesis (Iba et al., 2024).

One of the distinguishing properties of sepsis associated DIC is the positive feedback loop between coagulation activation and the intensity of the inflammatory response. Increased thrombin generation further amplifies inflammatory signaling pathways through protease activated receptors, leading to sustained cytokine production and additional activation of the coagulation system (Zhang et al., 2021). In addition, suppression of physiological anticoagulant systems and inhibition of fibrinolysis during sepsis prevent the clearance of accumulated fibrin, thereby aggravating microcirculatory impairment. These processes result in reduced tissue perfusion, cellular hypoxia, and organ failure, forming the biological basis for the strong association between DIC and high mortality in septic patients (Tsantes et al., 2023).

4. MICRORNAS AND THEIR RELATIONSHIP TO DIC

miRNAs represent small non-coding RNA species (~22 nt) involved in the post-transcriptional control of gene expression and negatively modulate protein synthesis by silencing target mRNAs. These molecules play central roles in critical biological processes such as immune responses, cellular stress, inflammation, endothelial function, and coagulation. miRNAs can be stably detected in both intracellular and circulating forms, making them attractive candidates as biomarkers and potential therapeutic targets (Antanokos et al., 2022). In sepsis studies, miRNA expression profiles have been associated with prognosis and disease severity. For instance, certain miRNAs are regulated in the context of inflammatory stimulation and influence immune cell activation. Others contribute to sepsis-associated coagulopathy by affecting endothelial cell homeostasis, platelet activation, and the synthesis of coagulation factors (Ho et al., 2016).

The role of microRNAs in the pathogenesis of DIC has been increasingly investigated, and several miRNAs have been demonstrated to exert significant regulatory functions at both clinical and experimental levels, particularly in sepsis-associated coagulopathy. These miRNAs constitute critical post-transcriptional regulatory checkpoints within the complex interplay between inflammatory and coagulation systems.

Among these, miR-19a-3p emerges as one of the most notable candidates. Studies have demonstrated that miR-19a-3p can suppress coagulation activity by directly or indirectly targeting tissue factor (TF) expression. In cases of sepsis-associated DIC, circulating levels of miR-19a-3p have been shown to be significantly reduced; conversely, experimental models in cell culture and animals have reported that upregulation of miR-19a-3p modulates TF expression as well as NF- κ B/AKT signaling pathways. These results indicate that miR-19a-3p may function as a critical anticoagulant regulatory molecule in the pathogenesis of DIC (Zhang et al., 2021).

miR-122-5p is another miRNA that has gained attention in studies of sepsis and sepsis-related coagulopathy. It has been reported to exert its effects through targets involved in complement and inflammatory pathways, such as MASP1, as well as oxidative stress response mediators like HO-1. Clinical studies provide evidence that serum miR-122-5p levels correlate with sepsis severity and coagulation abnormalities, and that this miRNA is significantly altered in patients developing DIC. These data suggest that miR-122-5p may serve as a potential biomarker reflecting both inflammatory and coagulation processes in sepsis-associated DIC (Wang et al., 2022).

miR-155, a well-known central regulator of inflammation, has been extensively studied in sepsis and systemic inflammatory models. Its close association with NF- κ B signaling pathways, as well as its effects on endothelial

cell activation and inflammatory cytokine production, has been well characterized. Increased miR-155 expression has been proposed to exacerbate endothelial dysfunction and promote procoagulant responses, thereby contributing to coagulation imbalance in sepsis. Accordingly, miR-155 is considered an important regulator of the inflammation–coagulation axis in DIC pathogenesis (Cánovas-Cervera et al., 2023).

In addition, miR-24 and miR-223 have been identified as miRNAs involved in processes such as PI3K/AKT signaling, platelet activation, and tissue factor regulation. In particular, the association of miR-223 with platelet function and immune cell activation represents an important example of the link between coagulation and inflammation. Although studies directly examining the relationship of these miRNAs with DIC remain limited, existing experimental evidence suggests that they may play indirect yet meaningful roles in DIC pathogenesis (Cánovas-Cervera et al., 2023).

Collectively, current literature indicates that inflammation, coagulation, and endothelial dysfunction are tightly interconnected at the molecular level in sepsis-associated DIC, and that microRNAs are key regulators within this complex network. miRNAs such as miR-19a-3p, miR-122-5p, miR-155, miR-24, and miR-223 emerge as potential molecular candidates that may contribute to DIC development through their roles in coagulation cascades, platelet activation, and inflammatory signaling pathways. However, the DIC-precise roles of these miRNAs, particularly in the early stages of the disease, endure insufficiently elucidated.

5. CONCLUSION AND FUTURE DIRECTIONS

Current evidence demonstrates that DIC represents a complex pathophysiological continuum in which inflammation, coagulation cascade activation, platelet consumption, and endothelial dysfunction are tightly interconnected. Within this multifactorial network, miRNAs have emerged as key post-transcriptional regulators that integrate these biological processes and may critically influence disease progression. The growing body of data indicates that specific circulating miRNA signatures are associated with disease severity and coagulation abnormalities, highlighting their potential value as mechanistic biomarkers in sepsis-related DIC.

From a translational perspective, the identification of DIC-specific circulating miRNA profiles may contribute to the development of novel biomarker candidates capable of reflecting early-stage disease biology, particularly when conventional coagulation assays are insufficient for timely detection. Integrating miRNA-based analyses with established clinical parameters, such as standard coagulation tests and the ISTH DIC scoring system, may enhance diagnostic accuracy and improve risk stratification in critically ill patients.

Future research should seek to uncover the molecular targets and gene modulatory networks of DIC-associated miRNAs in order to achieve a better understanding of the underlying disease mechanisms. In particular, defining miRNA-mediated regulatory pathways involved in endothelial dysfunction, thrombin generation, and immune–coagulation crosstalk will be essential for clarifying the molecular architecture of sepsis-induced coagulopathy.

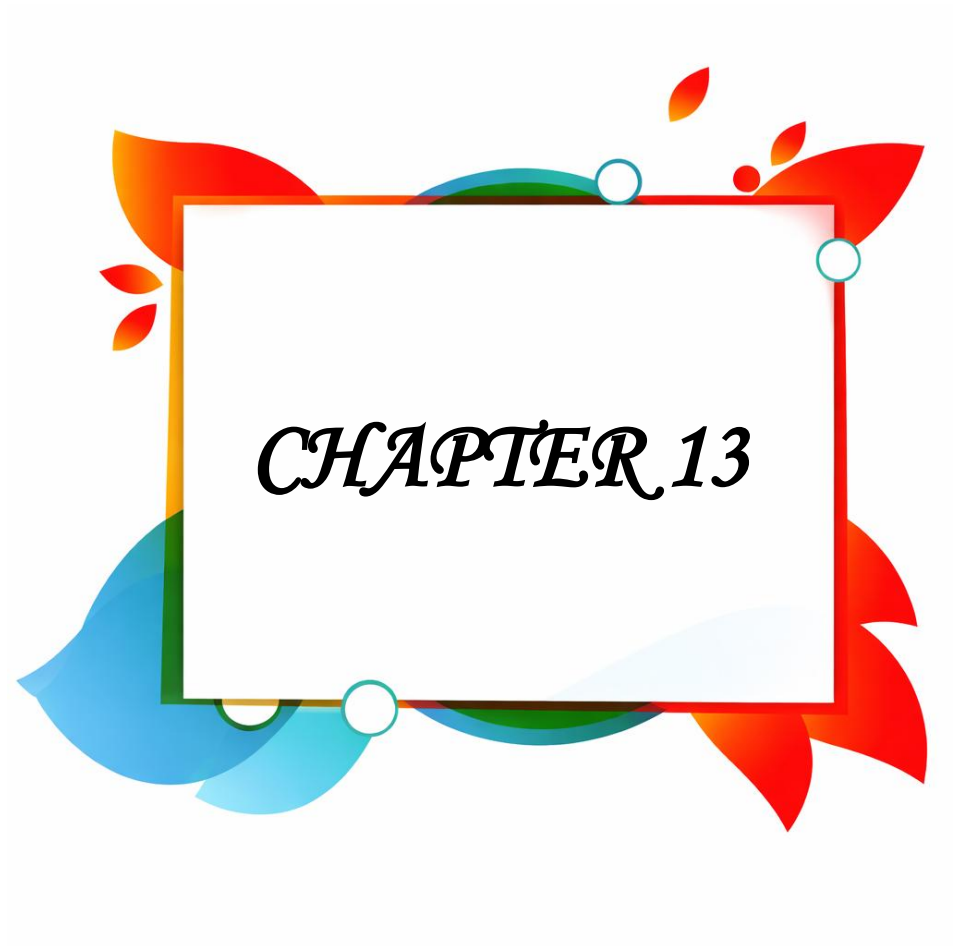
Moreover, integrated multi-omics approaches combining small RNA sequencing, bioinformatics analyses, and clinical datasets are expected to facilitate the discovery of novel miRNAs that have not yet been characterized in the context of DIC. Such discoveries may provide a foundation for the onset of next-generation diagnostic methods and miRNA-based therapeutic interventions targeting key regulatory nodes in the inflammatory–coagulation axis.

Importantly, the validation of candidate miRNAs in clinically well-defined cohorts will be critical for clinical translation. These efforts may ultimately enable the development of precision medicine approaches in sepsis-associated DIC, improving early diagnosis, prognostic assessment, and potentially guiding focused therapeutic interventions.

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Modern Perspectives on Next-Generation Antioxidants in Signaling Pathway Regulation

Mahira Firudin kizi Amirova¹

1. From Radical Scavengers to Redox Networks.

Antioxidants have often been introduced as compounds that protect cells by reacting with free radicals before they damage lipids, proteins, or nucleic acids [1,2]. This explanation is useful as a starting point, but it is too simple to describe how redox processes work in living systems [3]. In cells, oxidant production and antioxidant defense do not occur evenly throughout the cytoplasm [4,5]. They are shaped by where reactive species are produced, how quickly they react, which enzymes are nearby, what substrates are available, and how membranes organize local chemical reactions [6]. In this review, the author follows the transition from classical antioxidant concepts toward targeted and emerging antioxidant systems in contemporary redox biology [1,3]. Classical chain-breaking antioxidants, especially vitamin E and vitamin C, provide an important reference background for this transition because they illustrate membrane protection and antioxidant regeneration [7-9]. Particular attention is given to compartment-defined mechanisms, especially membrane-associated defenses involved in lipid peroxidation and ferroptosis [2,10-13]. Rather than presenting antioxidants only as general radical scavengers, the review treats them as parts of regulated protective networks that include antioxidant enzymes, thiol and redox cofactors, transporter-dependent metabolites, mitochondrial and membrane-localized defenses, and catalytic biomaterials [3-6,14,15]. The biological value of an antioxidant therefore depends not only on chemical reactivity, but also on localization, stability, regeneration, metabolic coupling, and relevance to a particular pathological process [2,5,6]. For this reason, newly recognized antioxidants are discussed in relation to their mechanisms, translational potential, and current limitations. By organizing these systems according to functional roles, this review aims to show how antioxidant research has moved beyond simple oxidative-stress models toward a more precise understanding of redox homeostasis, membrane protection, disease-specific oxidative injury, and

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targeted therapeutic design [1,2,6,14]. Hydrogen peroxide illustrates this point well. It participates in antimicrobial defense and other physiological reactions, but when levels are not controlled, it can contribute to biomolecular injury [3-5]. At lower and regulated levels, however, hydrogen peroxide can also act as a signaling molecule [4]. Superoxide has a similar dual role because it can participate in local redox signaling while also giving rise to more reactive oxidants [4,5]. Nitric oxide is even more context-dependent, since its effects vary with concentration, oxygen tension, metal availability, and inflammatory status [4,5]. For this reason, antioxidant molecules should be considered in relation to redox control rather than simple oxidant removal. Cells must limit harmful oxidation without eliminating redox signals needed for adaptation and normal physiological responses [3-6].

Recent interest in antioxidants has grown because redox biology is now studied with greater mechanistic resolution. Oxidative stress is increasingly examined in disease-specific contexts, including lipid peroxidation, mitochondrial dysfunction, inflammatory redox amplification, and ischemia-reperfusion injury [1,2,5]. Improved analytical methods have also made it easier to measure lipid hydroperoxides, oxidized sterols, and compartment-related redox changes [2,6]. These advances provide a clearer picture than older assays that measured oxidative stress only in broad, bulk terms [6,14]. New therapeutic concepts have emerged from this shift, particularly ferroptosis suppression [10,12,13,16-18], organelle targeting [14], and catalytic removal of reactive oxygen species by biomaterials [15,19-22]. Microbial and postbiotic research has added another route for antioxidant development, although biological effects depend strongly on preparation, standardization, and host context [23-25]. Recent nanozyme studies extend this catalytic logic to tissue repair and inflammation-related oxidative injury [26,27]. In parallel, fungal, marine, microbial, and food-chemistry pipelines are yielding antioxidant candidates with improved localization, stability, regeneration, or delivery compared with many classical compounds. Ergothioneine-related studies illustrate the fungal and dietary side of this trend [28-32]. Ovolthiols, mycosporine-like amino acids, seaweed-derived photoprotectants, food-derived peptides, and postbiotic systems extend the same logic to marine, food, and microbial sources [33-38]. Traditional antioxidants are summarized as a baseline in Table 1, where agents and systems are stratified according to their principal antioxidant roles [1-3,14].

Table 1. Classes of antioxidants in contemporary redox biology [1-3,14,15].

CLASS	REPRESENTATIVE EXAMPLES	CORE MECHANISM	TYPICAL LIMITATION
Direct radical-trapping antioxidants	Vitamin E, ergothioneine, ovoidiol, some flavonoids	Donate H or e- to terminate chain reactions	May be consumed stoichiometrically and require regeneration
Redox buffers and cofactor systems	Glutathione, thioredoxin, NADPH-linked systems	Maintain reducing environment and recycle oxidized species	Depend on metabolism and compartment access
Antioxidant enzymes	SOD, catalase, GPX, peroxiredoxins	Catalytically convert ROS to less reactive products	Localization and substrate specificity matter
Indirect antioxidants	Nrf2 activators, hormetic phytochemicals	Upregulate endogenous defense pathways	Dose-response can be nonlinear
Membrane-defense metabolites	CoQ10/ubiquinol, BH4, 7-DHC	Intercept lipid peroxy radicals or support ferroptosis resistance	Highly context- and membrane-dependent
Targeted antioxidants	MitoQ, SkQ1, peptide-guided antioxidants	Concentrate protection in organelles or tissues	Delivery, safety, and long-term retention remain issues
Microbial and food-derived antioxidants	Postbiotics, antioxidant peptides, polysaccharides	Mixed direct and indirect actions; may modulate gut-host redox axis	Bioavailability and standardization are variable
Catalytic antioxidant platforms	Nanozymes, artificial peroxidase/catalase mimics	Repeated ROS removal through enzyme-like activity	Translation requires toxicology, biodegradation, and manufacturing control

2. Reactive species relevant to antioxidants.

Not all reactive species are equally susceptible to antioxidant attack. Superoxide, hydrogen peroxide, hydroxyl radical, lipid alkoxyl radicals, lipid peroxy radicals, singlet oxygen, hypochlorous acid, nitric oxide, nitrogen dioxide, and peroxynitrite differ in lifespan, diffusion radius, target preference, and vulnerability to specific defenses [3-5]. These differences are important when antioxidant effects are interpreted in biological rather than purely chemical systems [6,14].

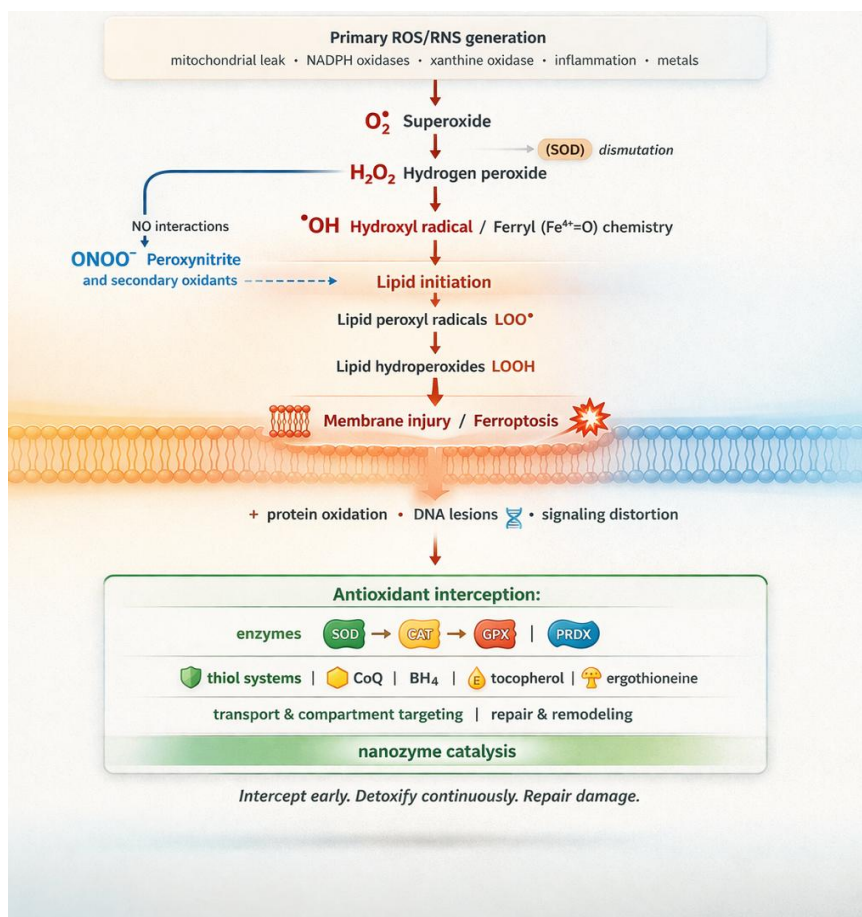


Figure 1. Oxidative damage and antioxidant interception scheme

The biological significance of a given antioxidant depends on which reactive species dominate in the relevant compartment. A water-soluble scavenger may have limited effectiveness against radical-chain reactions in membranes, whereas a lipophilic antioxidant may have little effect on peroxide-dependent signaling in the cytosol [2,7,8]. Membrane lipid peroxidation deserves particular attention because it links oxidative chemistry with membrane integrity, bioenergetic function, inflammation, and ferroptotic cell death [2,10-13]. Polyunsaturated phospholipids are especially susceptible to hydrogen abstraction, forming lipid radicals that rapidly react with oxygen to generate lipid peroxyl radicals and lipid hydroperoxides [2,11]. Once formed, these intermediates can continue damaging chain reactions unless they are neutralized, repaired, or removed by antioxidant systems [7,8,11]. For this reason, antioxidant enzymes and molecules that interrupt radical-chain propagation, support membrane remodeling, or clear oxidized lipids have become central to contemporary antioxidant research

[2,7,8,11-13]. A useful distinction is therefore not simply whether oxidants are produced, but whether their production remains physiologically regulated or becomes pathologically dysregulated. In healthy cells, hydrogen peroxide is continuously generated and degraded, and this controlled turnover contributes both to redox signaling and cellular defense [3,4]. Interventions that suppress peroxide formation non-selectively may weaken adaptive responses [3,4]. A more rational therapeutic approach is to target oxidative chemistry when it becomes damaging, for example during iron-catalyzed lipid peroxidation in ferroptosis or during the short oxidant burst associated with reperfusion injury [10-13,16,39]. Emerging antioxidants are therefore evaluated not only by radical-scavenging capacity *in vitro*, but also by selectivity, subcellular localization, compatibility with physiological signaling, and ability to reach the compartment where oxidative injury occurs [2,6,14].

3. Emerging Antioxidants vs. Classical

Any discussion of newly recognized antioxidants should begin with classical enzymatic and non-enzymatic systems, which still serve as mechanistic reference points. Superoxide dismutases catalyze the conversion of superoxide into hydrogen peroxide, while catalase and glutathione peroxidases contribute to detoxification of hydrogen peroxide and organic hydroperoxides [1,3]. Peroxiredoxins are also central because of their high reactivity toward peroxides and their role in peroxide-dependent signaling [3,4]. Glutathione- and thioredoxin-dependent pathways supply reducing equivalents for antioxidant enzyme recovery, redox repair, and maintenance of intracellular thiol balance [3-6]. Among dietary antioxidants, vitamins E and C remain classic examples of cooperative redox protection [7-9]. Vitamin E interrupts lipid-radical chain propagation in membranes, whereas vitamin C helps regenerate the tocopherol antioxidant pool within wider redox networks [7-9]. Coenzyme Q10 occupies a transitional position between classical and emerging antioxidant concepts [2,8]. It has long been recognized as a mitochondrial electron-transport component and, in its reduced ubiquinol form, as a lipophilic antioxidant [2,8]. Recent ferroptosis research has renewed its mechanistic importance because FSP1 can reduce coenzyme Q outside the classical GPX4-centered pathway, thereby supporting membrane-localized anti-ferroptotic defense [17,18]. This example shows that a known antioxidant may gain new biological significance when regulated cell-death research reveals an additional functional context [10,12,13,16-18]. Polyphenols, carotenoids, selenium-dependent enzymes, lipoic acid, uric acid, bilirubin, albumin, and endogenous sulfur-containing metabolites remain important parts of the conventional antioxidant repertoire [1,5,6,14].

The clinical history of classical antioxidants also shows why context matters. Many compounds perform strongly in *in vitro* antioxidant assays but do not produce consistent therapeutic benefits in clinical studies [1,5,14]. This discrepancy is often related to poor bioavailability, rapid metabolic turnover, insufficient delivery to the relevant subcellular compartment, or use in disease settings where broad supplementation does not match the dominant pathogenic mechanism [5,6]. These limitations have encouraged the current shift toward antioxidant strategies that are mechanistically targeted, network-informed, regenerable, or catalytic rather than defined only by reactivity in simplified chemical assays [10,13-16,19,21]. After the contrast between classical and emerging systems is established, it is important to clarify what “newly discovered” means.

4. “Newly Discovered Antioxidant” Concept

The term “novel antioxidant” should be used carefully. It may describe a recently isolated compound, a known molecule with a newly recognized cytoprotective role, or an older antioxidant reformulated through a delivery platform that changes its distribution or biological activity [10,12-14,16,39]. It may also refer to a metabolic or enzymatic network that has only recently been linked to disease pathogenesis, including the FSP1-CoQ10-NADPH axis described in ferroptosis resistance [10,12,13,17,18,40]. Ferroptosis research is especially important in this broader interpretation because it clarifies how endogenous membrane-protective systems regulate sensitivity to lipid-peroxidation-driven death [10-13,16]. Accordingly, emerging antioxidant molecules and systems include agents from non-traditional sources, such as ergothioneine, ovothiols, mycosporine-like amino acids, antioxidant peptides, postbiotic metabolites, and selected sterol intermediates [28,33,34,36,37,41,42].

Table 2. Newly discovered natural antioxidants from under-explored sources [23,24,28,33,34,36,37].

Antioxidant	Source domain	Main reported antioxidant logic	Current development direction
Ergothioneine	Fungi, bacteria, mushrooms	Stable sulfur antioxidant with dedicated transport	Foods, nutraceuticals, fermentation
Ovothiol A	Marine organisms, some algae-related systems	Highly reactive sulfur antioxidant; redox protection	Marine bioactive research and preclinical studies

MAAs	Algae, cyanobacteria, marine biomass	UV filtering plus antioxidant and anti-inflammatory action	Cosmeceuticals, photoprotection, biomaterials
Antioxidant peptides	Food proteins, fungi, enzymatic hydrolysates	Direct scavenging and pathway modulation	Functional foods and peptide discovery
Postbiotic metabolites/EPS	Microbial fermentation products	Gut-redox modulation, scavenging, immune regulation	Biotics, nutrition, gut-health formulations

Representative systems include the FSP1-CoQ10 axis, the GCH1-BH4 pathway, thioredoxin-associated defenses, melatonin and its downstream metabolite cascade, targeted antioxidant delivery platforms, and catalytic nanozymes [17,18,43-47]. This classification is useful because it avoids overstatement: not every recent report describes an entirely new chemical entity. It also separates direct antioxidant activity from indirect cytoprotection. Direct antioxidants chemically interact with radicals or oxidants, whereas indirect antioxidants enhance cellular defense by activating transcriptional programs, regulating metal availability, remodeling membrane composition, modifying metabolism, or reinforcing repair pathways [3,4,6]. In practice, many emerging candidates combine these mechanisms. Ergothioneine can react with damaging species, but its biological specificity is largely determined by OCTN1-mediated uptake and tissue accumulation [28-30,32,48]. OCTN1/SLC22A4-mediated transport means that ergothioneine does not simply diffuse into cells; rather, transporter activity supports its intracellular accumulation and may contribute to stress adaptation [28-30,48]. Molecular hydrogen may show selective reactivity toward highly damaging oxidants while also influencing redox-sensitive signaling pathways [49-51]. Postbiotic preparations likewise often combine scavenging chemistry with modulation of host defense pathways [23,24,37,38,52,53]. Table 3 summarizes these newly discovered or newly applied antioxidant categories and their functional activities.

Table 3. Newly discovered/recently applied antioxidants, their platforms, and functions [17-19,23,43,44,50].

Category	Definition	Example	Function
New molecule	Compound recently isolated or characterized	Novel peptides, marine thiols, rare polysaccharides	Expands chemical diversity of antioxidant toolbox
New function for known molecule	Previously known molecule with newly defined antioxidant role	CoQ10 in FSP1 defense, melatonin metabolites	Changes intracellular process without requiring a new chemical entity
New pathway/system	Recognition of integrated defense network	FSP1-CoQ-NADPH, GCH1-BH4, 7-DHC pathway	Improves disease-specific targeting
New delivery strategy	Targeted or stabilized deployment of antioxidant activity	Mitochondria-targeted antioxidants, nanoformulations	Addresses localization and bioavailability
New catalytic platform	Material that repeatedly removes ROS	Nanozymes, artificial peroxisome-like systems	Moves beyond stoichiometric scavenging

5. A Novel Antioxidant Ergothioneine

Recent biological evidence has highlighted ergothioneine as a biologically novel antioxidant, although the compound itself was already known and was historically described in relation to nitrogen-containing products in blood [28,48]. Table 4 summarizes the antioxidant features most relevant to its current interpretation: sulfur-based reactivity, transporter-mediated selectivity, chemical stability, and translational potential [28-32,48].

Table 4. The antioxidative properties of ergothioneine contributing to its translational relevance [28-32,48].

Feature	Ergothioneine profile	Implication
Chemical class	Sulfur-containing histidine derivative	Unusual redox chemistry compared with typical thiols
Source	Produced by selected fungi and bacteria; enriched in mushrooms	Supports food, fermentation, and biotech interest
Transport	Dedicated transporter OCTN1/SLC22A4	Enables tissue accumulation and biological specificity
Stability	Relatively stable and less prone to indiscriminate oxidation	May persist where labile antioxidants are depleted
Research trajectory	Growing interest in food, aging, neuroprotection, and inflammation	Strong candidate for translational nutrition and redox biology

Ergothioneine is a sulfur-containing histidine derivative with notable antioxidant properties. At physiological and slightly alkaline pH, it exists predominantly in the thione form, which contributes to its unusual stability and resistance to autoxidation. Under oxidative stress conditions, ergothioneine can participate in redox reactions by scavenging reactive oxygen and nitrogen species and by donating reducing equivalents when accepting hydrogen under acidic condition characteristic for inflammation state [28,32]. It is obtained mainly from selected fungi, bacteria, and mushrooms, with dietary and fermentation-derived sources receiving increasing attention [28,29,31]. Current literature distinguishes ergothioneine from glutathione and many other thiol-containing antioxidants because of its thione-thiol chemistry, chemical stability, favorable safety profile, and transporter-dependent intracellular accumulation [28-30]. Its entry into cells depends largely on the OCTN1/SLC22A4 transporter, so it should not be regarded merely as a passively circulating food-derived antioxidant [29,48]. This selective uptake helps explain why ergothioneine can accumulate in tissues where cytoprotection may be especially relevant [29,30,48]. Its mechanism is therefore better understood within an integrated antioxidant system that includes dietary supply, intestinal absorption, transporter-guided distribution, and enrichment in stressed tissues [28-30]. Such behavior may allow ergothioneine to remain available under conditions in which more labile antioxidants have already been

depleted [30,32]. Ergothioneine can interact with hydroxyl radicals, peroxynitrite-derived species, and metal-linked oxidants, but its physiological relevance extends beyond any single reaction rate [28,30,32]. Fermentation and biotechnological production have increased scientific interest in this compound because they expand its possible use in foods, nutraceuticals, and cosmetics [28,31,32]. Research on aging, inflammation, tissue resilience, and neuroprotection has further strengthened interest in stable sulfur-containing antioxidants such as ergothioneine [29,30,54]. Nevertheless, translational claims should remain cautious because human intervention data are still less extensive than mechanistic and preclinical evidence [29,30,54]. Thus, ergothioneine remains positioned between food-derived bioactive ingredients and redox-oriented therapeutic candidates. It may be considered a naturally derived antioxidant ingredient, an unusually stable sulfur-containing biomolecule, and a potential micronutrient-like factor, although its proposed conditional essentiality remains under debate [28-30,32].

6. Ferroptosis-Centered Antioxidants

Ferroptosis has shifted the discussion of antioxidants toward a more spatially defined problem: how cells restrain iron-dependent lipid oxidation before membrane damage becomes irreversible [2,10]. In this setting, the decisive events are not general ROS accumulation alone, but oxidation of polyunsaturated phospholipids and formation of lipid hydroperoxides within susceptible membranes [11,39]. This focus broadens antioxidant biology beyond vitamins, flavonoids, and other conventional dietary compounds [10,12,13]. Ferroptosis-oriented research emphasizes protection inside membrane microenvironments, where radical-trapping systems must be correctly localized to interrupt lipid-peroxidation cascades [11-13,16]. Lipid hydroperoxides formed through free radical-mediated lipid peroxidation are central mediators of ferroptotic damage, and appropriately positioned radical-trapping antioxidants can suppress this process [11-13,39] (Fig. 2).

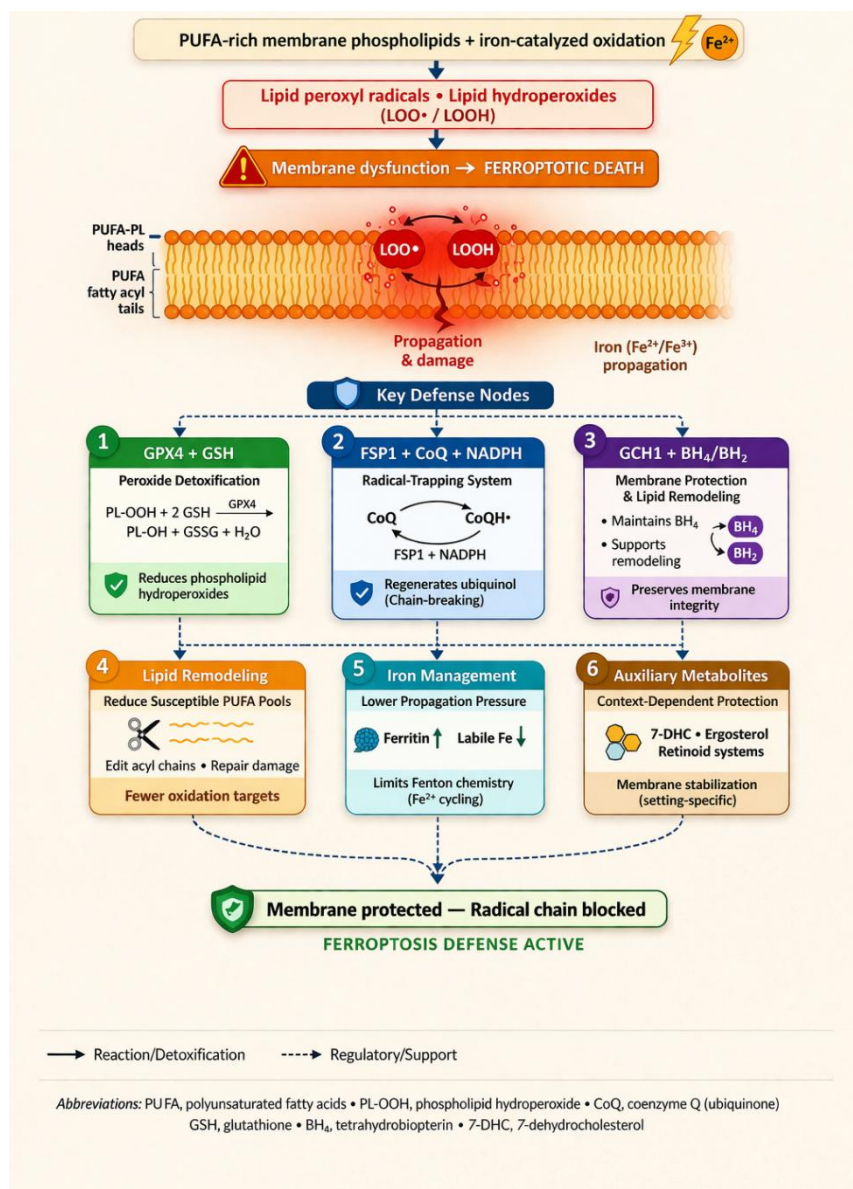


Figure 2. Antioxidants for defense against ferroptosis.

The main lesson from ferroptosis research is organizational rather than merely chemical. Cells resist ferroptosis through overlapping protective layers, including GPX4 supported by glutathione [11,12,39]. FSP1-dependent reduction of coenzyme Q provides a parallel membrane defense [17,18], while BH₄-linked protection connects ferroptosis resistance with lipid remodeling [43]. Regulation of iron availability remains central because iron chemistry drives lipid-peroxidation pressure [10,39]. Sterol-derived radical-trapping metabolites may

add another layer in selected settings [41,42]. This framework also improves terminology. Terms such as lipophilic chain-breaking antioxidant, anti-ferroptotic metabolite, membrane-localized redox defender, or radical-trapping lipid protector are often more precise than the broad label antioxidant [2,11,16]. In this field, discovery often means recognizing that a known metabolite or enzyme controls ferroptotic susceptibility in cells or animal models, rather than identifying a completely new chemical structure [10-13,16,39]. In other words, the chemistry may be familiar, while the disease-relevant redox context is newly defined [11-13]. The following subsections detail the principal membrane-defense axes introduced above and show how each contributes to ferroptosis resistance.

6.1 FSP1-CoQ10-NAD(P)H Antioxidant Defense Axis

Ferroptosis suppressor protein 1 (FSP1) has become an important example of how antioxidant defense can be organized outside the canonical GPX4 pathway [17,18]. Its relevance comes from its ability to reduce coenzyme Q and maintain a local ubiquinol pool capable of interrupting lipid peroxidation [17,18]. Recent work also places FSP1 among ferroptosis-resistance systems that depend on NADPH availability and cellular reductive metabolism [40]. Mechanistically, FSP1 uses NAD(P)H to convert ubiquinone into ubiquinol. The reduced ubiquinol pool functions in lipid-rich environments, where it can neutralize lipid peroxyl radicals and slow oxidative chain propagation [2,11]. This pathway is important because it may preserve membrane integrity when the GPX4-centered system is weakened [17,18]. Therapeutically, this means that failure of one antioxidant pathway may not determine cell fate if another membrane-protective axis remains active [13,16,40]. The compartmental dimension is also important, because FSP1 may protect selected membrane pools rather than the whole cell uniformly [17,40]. Consequently, antioxidant insufficiency should be assessed according to subcellular localization, especially in cancer, neurodegeneration, and ischemia-reperfusion injury [10,13,55-58].

6.2 Additional Ferroptosis-Related Membrane Defenses

Tetrahydrobiopterin (BH4) illustrates how a classical metabolic cofactor can acquire a newer antioxidant interpretation. Although BH4 is best known for its roles in aromatic amino acid hydroxylases and nitric oxide synthase, ferroptosis studies now place it within membrane-protective redox biology [43]. BH4 may contribute to radical-trapping activity and lipid remodeling, thereby lowering the probability that membrane oxidation reaches a lethal threshold [43]. This example is useful because it shows how rediscovered metabolic functions can reshape antioxidant science. It also explains why preservation of endogenous

cofactor pools may, in some settings, be more relevant than adding exogenous scavengers [43].

Sterol intermediates add another layer to this membrane-centered view. Recent work on 7-dehydrocholesterol suggests that it can influence ferroptosis sensitivity by donating hydrogen atoms to fatty-acid peroxyl radicals [41,42]. Related discussions of sterols indicate that ergosterol and other sterol structures may behave similarly in selected experimental settings [41,42]. These findings suggest that membrane composition itself can determine redox vulnerability. In cancer biology, this has implications for ferroptosis sensitivity, and more broadly it reinforces the idea that antioxidant defense is embedded within membrane ecology rather than acting as a separate chemical shield [2,11,41,42]. After membrane-centered ferroptosis defense, there is need to introduce antioxidants whose effects extend through hormone signaling, metabolite cascades, and mitochondrial regulation.

7. Melatonin-Driven Redox Protection and Metabolite-Mediated Antioxidant Defense

Melatonin broadens this framework because it is both a hormone and a redox-active regulator. Its antioxidant activity is not limited to direct radical scavenging; it also influences antioxidant enzymes and inflammatory tone [44,45]. Melatonin is also closely linked to mitochondrial performance and cardiolipin-related bioenergetic protection [59]. A further feature is its downstream metabolite cascade, which can continue redox protection after the parent molecule has reacted [45,46]. This makes melatonin different from many small antioxidants that are rapidly consumed after one or a few reactions [45,46]. In recent literature, melatonin is increasingly discussed in relation to neurodegeneration, developmental programming, mitochondrial resilience, and lipid-peroxidation control [44,47,60,61] (Fig. 3).

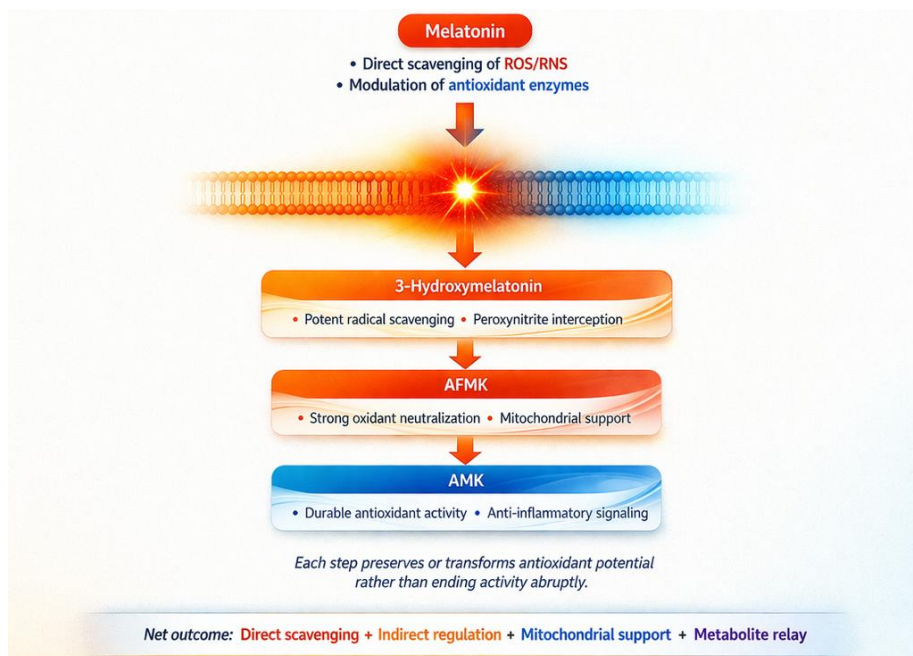


Figure 3. Melatonin-based sequential antioxidant defense.

The cascade concept is important because melatonin can be converted into several metabolites that still contribute to antioxidant defense [44,46]. These include cyclic 3-hydroxymelatonin, AFMK, and AMK, which have been associated with continued reactive-species neutralization and cytoprotective effects [45,46]. This sequential activity supports the description of melatonin as a multitiered antioxidant rather than a simple one-step scavenger [46]. In neurodegenerative and developmental contexts, melatonin is also evaluated as a mitochondria-relevant antioxidant and anti-inflammatory regulator [47,59-61]. A different emerging modality is represented by molecular hydrogen, which acts less like a conventional nutrient antioxidant and more like a diffusible redox modulator.

8. Molecular Hydrogen as a Selective Emerging Antioxidant Modality

Molecular hydrogen is now discussed as a selective but still methodologically challenging antioxidant modality [50]. Its small size allows diffusion across biological barriers, and it can be delivered through inhalation, hydrogen-rich water, saline, or topical preparations [49,50]. Unlike conventional nutrient antioxidants, hydrogen is not easily evaluated through standard food-chemistry assays such as DPPH or ABTS [49,52]. It is better described as a gaseous or physicochemical redox modulator. Some proposed effects involve direct interaction with highly damaging oxidants, including hydroxyl radicals and

peroxynitrite-related species [49-51]. Other effects may be secondary, involving inflammatory signaling, mitochondrial function, or antioxidant gene regulation [48,50]. For this reason, hydrogen should be presented cautiously: it is promising, but its mechanisms, dosing, endpoints, and clinical translation require careful standardization [49,50].

9. Food-Derived Peptides and Postbiotics as Antioxidants

Food-derived antioxidant peptides and postbiotic preparations represent a nutrition-oriented branch of emerging antioxidant research. Peptides released from food proteins, fungi, edible plants, or fermentation systems may scavenge reactive species, chelate metals, or influence endogenous defense pathways [36,62-68]. Their activity depends on sequence, hydrophobicity, amino acid composition, digestion stability, absorption, and interaction with the gut environment [36,68,69]. This makes peptide antioxidants difficult to rank by a single assay. A peptide that performs well in a chemical test may not show the same effect after digestion, transport, or metabolism [36,69]. Marine organisms and extremophile-adapted microbes provide a chemically diverse source of antioxidant candidates. Algae, cyanobacteria, marine invertebrates, and stress-tolerant microorganisms produce molecules that help them withstand UV exposure, salinity, desiccation, temperature shifts, and oxidative pressure [33,34,62-65]. Mycosporine-like amino acids are a good example because they combine UV-absorbing activity with antioxidant and anti-inflammatory potential [34]. Their relevance is particularly clear in skin protection and cosmeceutical research, where photoprotection and redox modulation overlap [34,35,63,67]. Ovolthiols represent another sulfur-containing marine antioxidant group whose chemical reactivity and evolutionary distribution have renewed interest in marine redox chemistry [33].

Postbiotics add a second mechanism-rich category because microbial metabolites, extracellular polysaccharides, cell-wall fragments, and fermentation products may influence both gut redox chemistry and host inflammatory signaling [23,24,52]. Some preparations combine direct scavenging with activation of host defense pathways such as Nrf2-related responses, while also affecting inflammatory mediators including NF- κ B-linked signaling [24]. The clinical and industrial appeal of postbiotics is that they may be easier to standardize than live probiotics, but composition, dose, production method, and target population still need careful definition [23,37,38,52,53]. The nutrition- and microbiome-based systems described above rely mainly on biological molecules; nanozymes, and targeted delivery platforms represent a more engineered strategy for controlling redox injury.

10. Antioxidant Nanozymes, Compartment- and Mitochondria-Targeted Antioxidants

Nanozymes represent a different type of antioxidant innovation. Rather than acting as single-use scavengers, these engineered materials are designed to imitate enzyme-like catalytic functions, such as superoxide dismutase-, catalase-, peroxidase-, or glutathione peroxidase-like activity [15,19-21]. This catalytic behavior may allow repeated ROS removal in environments where oxidant production persists [19,20]. Current designs include metal, metal-oxide, carbon-based, and Prussian blue-related platforms, each with different catalytic profiles and translational concerns [15,19-22].

Table 5. Catalytic nanomaterial platforms for redox protection [15,19-22,26].

Class	Typical catalytic mimicry	Examples	Main opportunity
Cerium-based	SOD-like, CAT-like redox cycling	Nanoceria, doped ceria	Inflammation and neuroprotection
Noble-metal based	Peroxidase/CAT/SOD-like depending on design	Pt, Pd-containing systems	High catalytic efficiency and tunable surfaces
Transition-metal oxides	Mixed antioxidant enzyme mimicry	Mn-, Fe-, Co-based materials	Cost and multifunctionality
Single-atom nanozymes	Highly defined active sites	Fe-N-C and related catalysts	Improved mechanistic precision
Hybrid or biomimetic systems	Multienzyme and responsive platforms	Artificial peroxisomes, coated composites	Disease-microenvironment adaptation

Note: Pt – Platinum, Pd - palladium

Compared with stoichiometric antioxidants, nanozymes may repeatedly detoxify reactive species, which is why they are often presented as catalytic antioxidant platforms [19-21]. This property could be useful in inflammatory and injury-related conditions where ROS production persists over time [19,20,26]. Nevertheless, the same catalytic activity that makes nanozymes attractive also raises safety questions. Translation requires attention to biodistribution, clearance, biodegradation, immune effects, long-term retention, and manufacturing reproducibility [21,22,27]. Thus, nanozymes should be described

as promising catalytic platforms rather than simple antioxidant supplements [15,21,22].

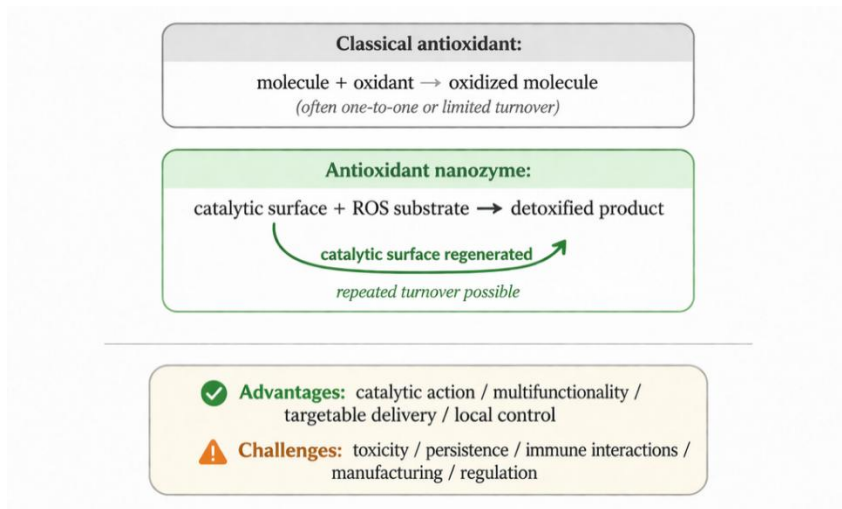


Figure 4. Distinguishing catalytic nanozyme antioxidants from classical stoichiometric scavengers.

Many unsuccessful antioxidant interventions can be explained, at least partly, by poor delivery to the compartment where oxidative injury actually occurs [1,2]. Mitochondria-targeted antioxidants, peptide-guided systems, liposomal carriers, and other delivery strategies attempt to solve this problem by concentrating antioxidant activity in organelles, membranes, or tissues where redox injury is most relevant [6,14]. This logic is especially important in mitochondrial dysfunction and ischemia-reperfusion injury, where oxidative damage can arise in short, localized bursts [1,5,13]. Targeting can increase biological plausibility, but it also introduces new questions about retention, dose, off-target effects, and long-term safety [5,14]. Because these antioxidant classes differ widely in chemistry, localization, and intended use, their evaluation requires assay choices that match mechanism and biological compartment.

11. Antioxidant Assays, Interpretation, and Ranking

Antioxidant testing remains difficult because many assays measure chemical reactivity under simplified conditions rather than biological protection in a living system [6,14]. DPPH, ABTS, ORAC, FRAP, and related tests can be useful for screening, but they do not adequately capture compartmentalization, metabolism, regeneration, membrane access, or signaling compatibility [6]. For lipid peroxidation and ferroptosis, assays that monitor lipid hydroperoxides, oxidized phospholipids, cell viability, iron dependence, and rescue by established

ferroptosis inhibitors are more informative [2,10-13]. For food-derived or microbial antioxidants, digestion stability and bioavailability are central considerations [36-38,53,68,69]. For postbiotic preparations, batch composition and standardization are especially important [23,37,52]. For nanozymes, catalytic kinetics, substrate specificity, and toxicological behavior must be evaluated together [15,19-22].

Table 6. Interpretive strengths and limitations of antioxidant testing methods [6,14,19,21,36,49,62,69].

Assay family	Captures well	May miss
Colorimetric radical assays	Fast electron/H-atom transfer in simplified media	Localization, metabolism, membrane protection, catalytic turnover
Reducing power assays	General reducing potential	Species specificity and compartment biology
Lipid oxidation models	Chain-breaking capacity in lipid systems	Transport, tissue uptake, long-term adaptation
Cellular ROS probes	Intracellular redox trends	Precise oxidant identity and kinetic detail
Disease models	Functional relevance	Mechanism can remain ambiguous without orthogonal assays

The path to translation differs markedly among emerging antioxidant classes. Ergothioneine is being developed mainly through food, nutraceutical, and fermentation-based routes, supported by its dietary origin and favorable safety profile [28-31]. Molecular hydrogen is usually positioned as a nonconventional redox modality, but its clinical interpretation depends on delivery format, dose, and disease-specific endpoints [49-51]. Melatonin already has a long biological and pharmacological history, while its metabolite cascade and mitochondrial actions support renewed antioxidant interest [44-47,60,61]. Nanozymes and targeted antioxidant platforms follow a more drug-like development pathway because their material properties, biodistribution, clearance, and safety profile must be defined rigorously [15,19-22,27]. Thus, a food-derived ingredient may advance through safety documentation and cautiously framed functional claims, while an intravenously administered catalytic material must meet a pharmacological standard of evidence. This distinction is important because antioxidant terminology can make very different products appear comparable. In

practice, each class should be judged according to its mechanism, route of delivery, exposure level, target tissue, and intended use [1-4,14,15].

12. Comparative Classification of Antioxidants

12.1 Direct Antioxidants Versus Indirect

Antioxidant activity is better viewed as a spectrum than as one uniform mechanism. At one end are direct antioxidants that chemically intercept radicals or oxidants, a role that may be critical during short bursts of lipid peroxidation or oxidant overload [7-9,11]. At the other end are indirect antioxidants that strengthen endogenous defenses by changing gene expression, metabolism, metal handling, or repair capacity [3-6]. Many emerging compounds fall between these categories because they combine chemical reactivity with transporter-dependent localization, pathway modulation, or metabolic support [28,30,43-45,49,52].

Timing also matters. A rapidly acting radical trap may be most useful during acute oxidative injury, whereas an indirect modulator may be more appropriate when chronic inflammation or metabolic imbalance reshapes the redox environment over time [1,5]. This is why the same antioxidant can appear protective in one model and weak or even poorly matched in another. Mechanism, disease stage, dose, and compartmental access must be considered together [6,14].

12.2 Comparative Note on Hydrophilic, Lipophilic, Nutritional, and Pharmacological Antioxidants

Hydrophilic and lipophilic antioxidants protect different biological spaces. Water-soluble systems, including glutathione, thioredoxin-related pathways, and vitamin C, are most relevant in cytosolic, extracellular, or plasma environments [3-6,9]. Lipid-soluble antioxidants such as tocopherols, ubiquinol, and selected membrane-active metabolites protect lipid-rich domains and phospholipid bilayers [2,7,8]. Ferroptosis makes this distinction especially important because the lethal process occurs within oxidizable membrane lipids [10-13,16,39].

Nutritional and pharmacological antioxidants should not be evaluated by the same expectations. In nutrition, antioxidant compounds are usually assessed for long-term dietary exposure, safety, bioavailability, and support of physiological resilience [28,52,53]. Pharmacological antioxidants, in contrast, must show disease-specific efficacy, appropriate delivery, dose-response behavior, and acceptable safety under therapeutic conditions [1,5,14]. This distinction is important for compounds such as ergothioneine, hydrogen, melatonin, and nanozymes, which occupy different positions between food chemistry, redox biology, and therapeutic development [28,44,45,49-51].

13. Biomarker Strategy, Sustainability, and Manufacturing of Antioxidant Systems

Total antioxidant capacity is too nonspecific to support modern mechanistic claims [6,14]. More informative biomarkers may include lipid hydroperoxide patterns, oxidized phospholipid panels, F2-isoprostanes, glutathione redox status, thioredoxin/peroxiredoxin changes, mitochondrial readouts, or ferroptosis-related markers [2,6,10-13]. Biomarker selection should match the proposed mechanism. For example, a membrane-protective antioxidant should be linked to lipid-peroxidation endpoints, whereas a postbiotic should also be evaluated through inflammatory and gut-related markers [2,23,24,52].

Sustainability is becoming part of antioxidant selection, especially for ingredients intended for repeated use in foods, cosmetics, or nutraceuticals [28,31]. Fermentation-derived ergothioneine, peptide production from food side streams, and marine-biomass sourcing of MAAs all connect antioxidant development with circular bioeconomy strategies [31,34,36,69]. These approaches are attractive because they may reduce waste and improve production scalability, but they also require quality control, standardized composition, and safety evaluation [23,36-38,52,53].

Table 7. Comparison of emerging antioxidants [19,28,33,34,36,37,43,44,49,50].

Entity	Primary type	Key strength	Main caution
Ergothioneine	Specialized dietary sulfur antioxidant	Transport-enabled tissue accumulation	Clinical efficacy still developing
FSP1-CoQ system	Endogenous membrane defense pathway	GPX4-independent anti-ferroptotic protection	Pathway dominance is context dependent
BH4-related defense	Metabolite-centered endogenous pathway	Links metabolism to membrane protection	Complex cofactor biology
7-DHC / sterol defense	Newly appreciated membrane radical trap	Reframes membrane composition as antioxidant variable	Not a simple supplementation story
Melatonin cascade	Endogenous multitier antioxidant system	Direct + indirect + metabolite relay	Broad claims require careful context

Entity	Primary type	Key strength	Main caution
Molecular hydrogen	Selective redox-modulating modality	High diffusibility and apparent safety	Mechanistic and trial heterogeneity
Ovothiols	Marine sulfur antioxidants	Strong chemical reactivity and novelty	Limited translational maturation
MAAs	Photoprotective multifunctional antioxidants	UV shielding plus antioxidant value	Application often domain-specific
Antioxidant peptides	Food-derived functional bioactives	Tunability and AI-assisted discovery	Bioavailability varies
Postbiotics	Mixture/system-level antioxidants	Formulation stability and gut-host relevance	Standardization remains difficult
Nanozymes	Catalytic antioxidant platforms	Repeat turnover and engineering flexibility	Safety and regulation are major hurdles

Fermentation-derived ergothioneine, valorization of food side streams for antioxidant peptides, and marine-biomass production of MAAs illustrate how antioxidant development can overlap with circular bioeconomy strategies [36,62,65,69]. These approaches are promising, but sustainability claims should be supported by production data, quality control, and reproducible biological characterization [23,36-38,52,53].

Table 8. Classification of antioxidants by translational maturity [1,14,17,19,28,33,34,36,37,43,44,49,50,58].

Group	Examples	Mechanistic maturity	Translational maturity
Well-established classical systems	SOD, CAT, GPX, vitamin C/E	Very high	High to moderate
Newly appreciated endogenous systems	FSP1-CoQ, BH4, 7-DHC-linked defense	High and increasing	Early to moderate
Emerging dietary/natural compounds	Ergothioneine, ovothiols, MAAs	Moderate to high depending on compound	Early to moderate

Group	Examples	Mechanistic maturity	Translational maturity
Food and microbiome-derived candidates	Peptides, postbiotics, EPS	Moderate	Early to moderate
Platform technologies	Targeted antioxidants, nanozymes	Variable but rapidly improving	Mostly early

After the mechanistic and translational comparison, it is useful to examine where these systems may be most relevant in disease contexts.

14. Disease-Oriented Protective Perspectives on Emerging Antioxidants

A disease-oriented perspective has helped the antioxidant field move beyond generic oxidative-stress language [1,5,14]. Neurodegeneration, cardiovascular injury, metabolic disease, mucosal inflammation, skin photoaging, and cancer each involve different redox pressures, tissue barriers, and therapeutic risks [5,10-13]. Therefore, the same antioxidant may be protective in one context, neutral in another, and potentially undesirable in a third [70-72].

Neurons are especially vulnerable to oxidative injury because they are long-lived, metabolically active, lipid-rich, and relatively limited in regenerative capacity [47,61]. Aging and neurodegenerative disease add further stress through mitochondrial dysfunction, iron imbalance, chronic inflammation, impaired proteostasis, and altered lipid metabolism [5,47,61]. These features explain why neuroprotection is often discussed in relation to mitochondrial antioxidants, melatonin, ergothioneine, and ferroptosis-limiting systems [10,12,13,44,47,61].

Ferroptosis-limiting mechanisms are increasingly relevant in Parkinsonian, retinal, and broader neurodegenerative models, where iron handling and phospholipid oxidation influence cell vulnerability [13,56]. Ergothioneine, melatonin, and selected ferroptosis-related metabolites are therefore discussed as candidates for neuronal redox resilience [28-30,43,44,47,61]. However, clinical translation still requires stronger human evidence, careful biomarker selection, and disease-specific trial design [1,14,54].

Cardiovascular tissues encounter oxidative pressure during atherosclerosis, endothelial dysfunction, hypertension, metabolic overload, and especially ischemia followed by reperfusion [1,5,13]. Reperfusion produces a short but intense oxidant surge, so timing and compartmental delivery are critical [13].

Antioxidants that target mitochondrial dysfunction, inflammatory redox amplification, or membrane lipid peroxidation may be more plausible than nonspecific supplementation [2,5,13]. Postbiotic and microbial-metabolite approaches are also being discussed in relation to cardiometabolic and post-ischemic remodeling pathways [23-25].

Metabolic disorders involve more than excess oxidants. Obesity, diabetes, and related conditions combine nutrient overload, mitochondrial inefficiency, inflammation, lipotoxicity, endothelial dysfunction, and changes in the gut microbiome [5]. Antioxidant approaches may therefore be more meaningful when they improve metabolic-redox coordination rather than simply increasing total antioxidant capacity [3,5,6]. This is one reason postbiotics, food-derived peptides, ergothioneine, and mitochondrial redox modulators are being explored in metabolic contexts [23,24,28,36,37,49,52,53].

The gastrointestinal tract is a particularly active site for antioxidant research because it connects diet, microbiota, immune activity, epithelial renewal, and chemically reactive luminal contents [23,24,52]. Postbiotics and food-derived peptides may influence mucosal redox balance through local scavenging, barrier support, immune modulation, and activation of endogenous defense pathways [24,36-38,53]. However, translation depends on preparation standardization, dose, matrix, and clinically relevant inflammatory or oxidative-stress endpoints [23,38,52,53].

Cancer is one of the most context-dependent areas for antioxidant intervention. Antioxidant protection may reduce treatment-related tissue damage in some settings, but it may also protect malignant cells or interfere with therapies that rely on oxidative stress [70-72]. Ferroptosis adds another layer because some cancer strategies aim to increase, not suppress, lipid peroxidation [57,58]. Therefore, anti-ferroptotic antioxidants may be protective for normal tissues in one setting but undesirable in tumors where ferroptosis induction is a therapeutic goal [57,58,70,71].

Skin and exposed epithelia face UV radiation, pollution, inflammation, lipid oxidation, and age-related redox decline [34,35]. This makes multifunctional antioxidants attractive for photoprotection and cosmeceutical development. MAAs combine UV-screening and antioxidant properties, while seaweed-derived compounds and marine metabolites may support anti-photoaging strategies [62-64,66,67]. Ergothioneine is also of interest in cosmetic and skin-related applications because of its stability and biological transport profile [28-30,32].

Table 9. Emerging antioxidants in selected disease-related and undesirable redox conditions [13,17,19,23,28,33,34,36,37,43,44,49,50,57,70].

Emerging antioxidant	Strongest current rationale	Action mechanism	Principal unresolved issue
Ergothioneine	Aging, neuroprotection, chronic inflammation, nutrition	Stable sulfur antioxidant with transporter-mediated tissue uptake	Dose-response and intervention endpoints in humans
FSP1-CoQ / anti-ferroptotic systems	Retinal injury, kidney injury, neurodegeneration, reperfusion	Direct relevance to membrane lipid peroxidation and ferroptosis	May be undesirable in some cancers
Melatonin cascade	Neurodegeneration, sleep-linked oxidative states, inflammation	Direct + indirect action with mitochondrial relevance	Optimal dosing and context specificity
Molecular hydrogen	Reperfusion injury, metabolic stress, skin, adjunctive medicine	Rapid diffusion and selective redox modulation	Heterogeneous protocols and mechanisms
MAAs	Skin photoprotection and topical systems	UV filtering plus antioxidant multifunctionality	Systemic applications remain less developed
Postbiotics / EPS	Gut and metabolic diseases, inflammatory mucosal disorders	Barrier, immune, microbial, and redox crosstalk	Standardization of composition
Nanozymes	Acute inflammatory injury, reperfusion, localized tissue damage	Catalytic and targetable ROS removal	Toxicology, biodegradation, regulation

The disease-oriented discussion can be condensed into a classification atlas that organizes antioxidants by origin, compartment, and mechanism.

15. Antioxidant Classification Atlas

Antioxidants are best understood as a diverse set of molecules, enzymes, metabolites, transport systems, and materials that alter the probability and consequences of oxidative injury [1-6]. Their classification should therefore consider source, chemistry, cellular localization, mechanism, disease relevance, and translational maturity [2,6,14]. This atlas-like view avoids reducing

antioxidants to a single radical-scavenging score and instead places them within biological networks [3,4].

Several priorities follow from this view. Human studies are needed to clarify the clinical relevance of ergothioneine and hydrogen-based interventions [28-30,49-51]. More standardized preparation and characterization are necessary for postbiotics and antioxidant peptides because their effects depend heavily on composition, production method, and digestion or matrix behavior [23,36-38,52,53]. MAAs require similar attention to source, purity, photoprotective activity, and formulation context [34,35,62-64,66,67]. Nanozymes need especially careful evaluation of catalytic behavior, biodistribution, degradation, and long-term safety [15,19-22]. Ferroptosis-related antioxidants require disease-specific evaluation because suppressing ferroptosis may be helpful in degenerative or ischemic injury but potentially harmful in cancers where ferroptosis induction is desirable [10,12,13,57,58,70-72].

In summary, the modern antioxidant field has moved from a simple radical-scavenger model toward a more precise framework based on compartmental redox control, membrane protection, catalytic detoxification, and disease-oriented targeting [1-6,11,13-15]. Ergothioneine, ferroptosis-related membrane defenses, melatonin metabolites, molecular hydrogen, marine photoprotectants, postbiotics, antioxidant peptides, and nanozymes illustrate this transition from general chemical reactivity to context-dependent biological function [37,43,44,49-52]. The strongest translational claims will come from studies that connect mechanism, localization, biomarker change, safety, and clinical outcome in the same experimental design [1,6,14].

Table 10. Antioxidant classification by source of origin [19,28,33,34,36,37,41,49,50,61].

Origin class	Representative antioxidants	Distinctive feature
Endogenous human/animal metabolites	Melatonin, bilirubin, uric acid, BH4, coenzyme Q	Integrated into host metabolism and regulation
Diet-derived natural compounds	Vitamin C, polyphenols, carotenoids, ergothioneine	Acquired largely from food and modified by bioavailability
Marine and extremophile compounds	Ovothiols, MAAs, unusual polysaccharides	Adapted to UV, salinity, and unusual ecological stresses
Microbial products and postbiotics	EPS, organic acids, cell-wall fragments, microbial antioxidants	Systems-level effects through host-microbe interactions

Origin class	Representative antioxidants	Distinctive feature
Food-derived bioactive fragments	Antioxidant peptides and hydrolysates	Tunable sequences and side-stream valorization
Synthetic small molecules	Designed radical traps, organelle-targeted analogs	Chemically optimized for potency or localization
Engineered materials	Nanozymes, biomimetic catalytic particles	Catalytic rather than stoichiometric protection

Table 11. Antioxidant classification by dominant compartment [2,3,17,19,28,33,34,36,37,41,49,50,61].

Compartment	Antioxidants	Action
Plasma / extracellular fluid	Vitamin C, urate, albumin-associated defenses	Important but not sufficient for tissue protection
Cytosol	Glutathione, thioredoxin-linked systems, some peptides	Central to buffering and enzyme regeneration
Mitochondria	CoQ, melatonin, targeted quinones, some nanoformulations	High-value target in energy-rich tissues
Membranes / lipid droplets	Vitamin E, ubiquinol, FSP1 system, BH4-related defense, sterol intermediates	Critical in ferroptosis and lipid peroxidation
Gut lumen / mucosa	Postbiotics, polysaccharides, peptides, hydrogen-rich preparations	Local action may matter more than systemic absorption
Skin surface / epidermis	MAAs, ergothioneine formulations, topical hydrogen systems	Photodamage and pollution create mixed oxidative challenges

Table 12. Antioxidants classification by action mechanism [2,3,7,14,17,19,37,43,44,50,57].

Mechanistic class	Examples	Action mechanism
Chain-breaking radical trap	Vitamin E, ubiquinol, 7-DHC in specific contexts	Stops propagation of radical chain reactions
Peroxide detoxification	Catalase, GPX, PRDX, CAT-like nanozymes	Converts reactive peroxides to safer products
Thiol or redox-buffer support	GSH, thioredoxin, ergothioneine partly	Maintains reducing environment and recycling
Metal management	Ferritin-linked systems, chelators, albumin contributions	Lowers catalytic pressure for ROS formation
Signaling transcriptional adaptation /	Nrf2 activators, some polyphenols, postbiotics	Raises endogenous defense capacity
Membrane remodeling or susceptibility lowering	BH4 pathway, lipid remodeling enzymes	Makes membranes less easy to oxidize
Catalytic defense artificial	Nanozymes, artificial peroxisomes	Repeated turnover by designed materials

The chapter can therefore be summarized through the central shift from nonspecific scavenging to targeted redox-system control.

15. Limitations, Controversies, and Prospects for Antioxidant Application

Issues described above lead directly to the main limitations and controversies in antioxidant application. The central controversy in antioxidant research is no longer whether oxidative processes contribute to disease. The more difficult question is how to modulate harmful oxidation without suppressing redox signals required for adaptation, immunity, repair, and normal metabolism [3,4]. Broad supplementation can be ineffective or even misleading when it is not matched to the dominant mechanism of injury [1,5,14]. Future progress will therefore depend on matching antioxidant type to disease context, compartment, timing, and measurable biomarker change [2,6,14]. To integrate the preceding sections, the following comparison separates antioxidants by mechanism, solubility, development route, and biomarker strategy.

16. Chapter Summary

Antioxidants are no longer understood simply as small molecules that quench free radicals in isolation. Contemporary redox biology places antioxidant action within networks shaped by compartmentalization, metabolism, membrane structure, transport, regeneration, and disease context. This shift is especially clear in ferroptosis, where protection depends on coordinated membrane defenses such as GPX4, FSP1-CoQ10, BH4-related pathways, lipid remodeling, iron control, and sterol-linked radical trapping. Emerging candidates, including ergothioneine, melatonin, molecular hydrogen, marine antioxidants, food-derived peptides, postbiotics, targeted platforms, and nanozymes, illustrate how antioxidant science now spans nutrition, pharmacology, biotechnology, and materials research. Their translational value will depend on precise mechanisms, appropriate biomarkers, careful safety evaluation, and human evidence rather than broad antioxidant claims alone.

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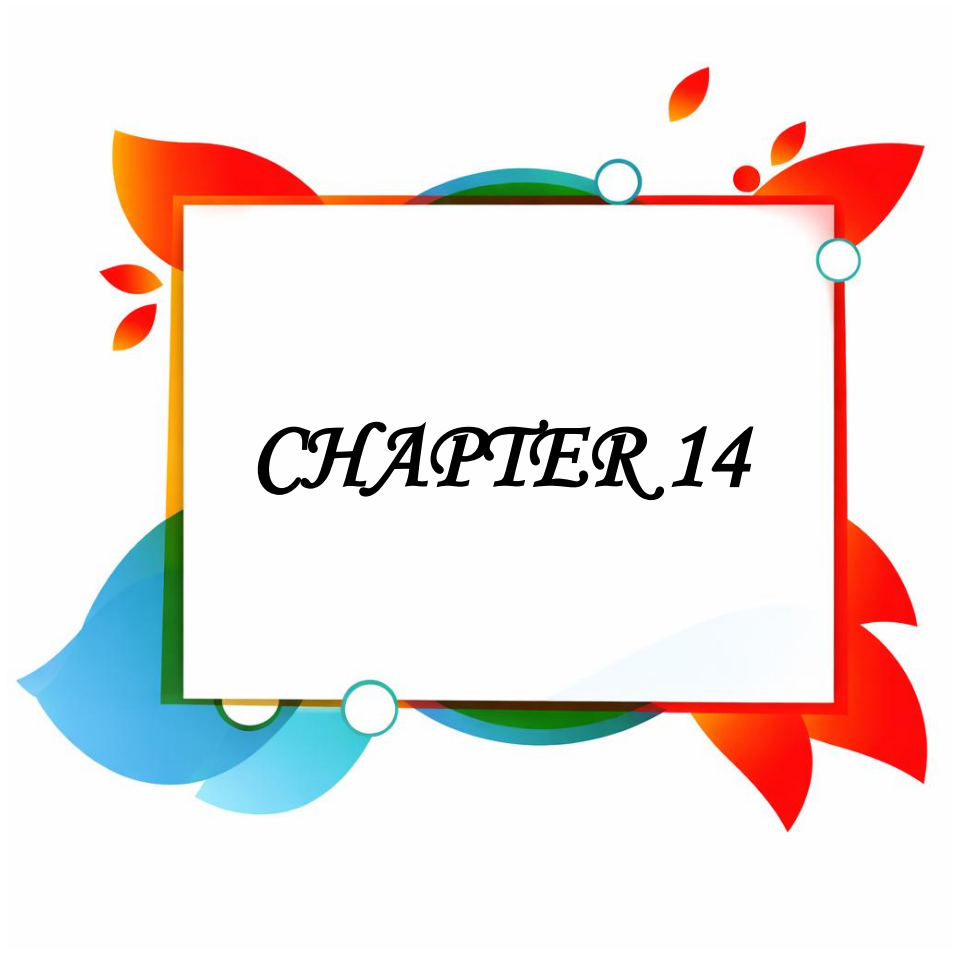
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Inhibition of Cancer-Related Enzymes with Heavy Metals

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Introduction

Cancer is a multifactorial and complex group of diseases characterized by the uncontrolled regulation of cellular growth and proliferation. During cancer development, significant alterations occur in cellular metabolism, energy production, and signal transduction pathways. Enzymes play a central role in the regulation of these processes by catalyzing biochemical reactions that are essential for the maintenance of cellular viability. Alterations in enzyme activity can disrupt normal cellular physiology, thereby contributing to tumor initiation, progression, and metastasis. In particular, metabolic reprogramming observed in cancer cells is characterized by profound changes in glucose metabolism, lipid biosynthesis, and amino acid utilization. One of the most prominent examples of these metabolic alterations is the “Warburg effect,” in which cancer cells preferentially convert glucose into lactate even in the presence of sufficient oxygen.

Heavy metals are naturally occurring elements that can exert diverse effects on biological systems. While certain metals, such as copper, iron, zinc, and selenium, function as essential trace elements at low concentrations, others, including cadmium, mercury, and lead, can induce toxic effects even at relatively low levels of exposure. The toxicological effects of heavy metals include the generation of oxidative stress, DNA damage, disruption of cellular redox homeostasis, modulation of apoptotic pathways, and inhibition of metabolic enzymes. These effects may impair cellular homeostasis and contribute to cancer initiation and progression. However, not all heavy metals exert exclusively toxic effects. Essential trace metals such as copper (Cu), zinc (Zn), iron (Fe), selenium (Se), and molybdenum (Mo) play important roles in the regulation of cellular metabolism when present at physiological concentrations. These elements contribute to the maintenance of cellular homeostasis by supporting antioxidant defense systems, reducing oxidative stress, and regulating DNA synthesis and

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repair processes. Consequently, appropriate levels of certain trace metals have been reported to exert protective effects against carcinogenesis.

Enzyme inhibition represents one of the major mechanisms involved in the regulation of enzymatic activity. Heavy metals can interact with proteins, particularly through binding to sulfhydryl groups, resulting in conformational alterations that impair enzymatic function. Such interactions may disrupt cancer-associated metabolic pathways and interfere with essential cellular processes. Recent studies have demonstrated that the effects of heavy metals on cancer-related enzymes are important not only from a toxicological perspective but also for understanding cancer biology and identifying novel therapeutic targets. Therefore, this chapter discusses cancer-related enzymes, the biological effects of heavy metals, and the inhibitory actions of heavy metals on these enzymes in light of current scientific evidence.

Cancer-Related Enzymes

Enzymes are proteins that catalyze a wide range of biochemical reactions in living organisms (Onyiba et al., 2025). Alterations in these processes can transform the normal structure and function of cells. Transformed cells adapt to the resulting metabolic changes, enabling their survival and contributing to the initiation and progression of tumors (Hanahan & Weinberg, 2011).

Cancer cells are characterized by several hallmark features, including sustained proliferative signaling, evasion of growth suppressors, resistance to cell death, replicative immortality, induction of angiogenesis, and activation of invasion and metastasis. These characteristics facilitate uncontrolled cellular proliferation and the spread of cancer cells into surrounding tissues (Rashid, 2017). Cancer progression is closely associated with the reprogramming of cellular signaling pathways, disruption of cellular energy homeostasis, and metabolic adaptations that meet the altered bioenergetic demands of rapidly proliferating cells (Hanahan & Weinberg, 2011). One of the most prominent metabolic reprogramming mechanisms associated with cancer cell proliferation is aerobic glycolysis, commonly known as the Warburg effect. In normal cells, glucose is metabolized to pyruvate through glycolysis, after which pyruvate enters the tricarboxylic acid (TCA) cycle and generates ATP through oxidative phosphorylation. In contrast, glucose metabolism follows a different route in most tumor cells. Even in the presence of oxygen, pyruvate is rapidly converted into lactate by lactate dehydrogenase (LDH), a phenomenon referred to as the “Warburg effect” (DeBerardinis et al., 2008; Jin et al., 2016; C. L. Lu et al., 2015; J. Lu et al., 2015).

In addition, glucose taken up by cancer cells is redirected toward various anabolic processes, including ribose production, amino acid biosynthesis, and protein glycosylation. Recent studies have demonstrated that malignant transformation is not limited to aerobic glycolysis alone but also involves

multiple metabolic reprogramming pathways, including lipid biosynthesis and lipid metabolism, the pentose phosphate pathway (PPP), and increased glutamine utilization (Jiang et al., 2014; Schulze & Harris, 2012; Wakil & Abu-Elheiga, 2009).

Heavy Metals

Heavy metals are elements characterized by relatively high atomic weight, atomic number, and density (Järup, 2003). Based on their environmental and biological effects, heavy metals can be classified into four categories. Category I includes highly toxic heavy metals such as lead, mercury, and cadmium, which pose significant threats to human health and the environment; therefore, their use and emissions must be strictly controlled. Category II comprises moderately toxic heavy metals, including chromium, cobalt, and manganese, which are harmful to both human health and ecological systems and require continuous monitoring. Category III consists of low-toxicity heavy metals such as copper (Cu), zinc (Zn), and iron (Fe), which are generally less harmful but may exert adverse environmental effects when accumulated excessively. Category IV includes trace heavy metals such as molybdenum (Mo), vanadium (V), antimony (Sb), and related elements, which pose minimal risk to human health and ecosystems but still require monitoring and regulation (Cheng et al., 2025). Heavy metal ions can function as both cofactors and inhibitors in enzymatic pathways (Witkowska et al., 2021). While some of these metals serve as essential trace elements required for the maintenance of biological processes, others are considered biologically inert and relatively harmless. However, certain heavy metals can exert severe toxic effects even at very low concentrations (Rahman et al., 2019). Heavy metals frequently lose one or more electrons and react with biological systems by forming metal cations that exhibit a strong affinity for nucleophilic sites within vital macromolecules. The acute and chronic toxic effects of heavy metals can affect multiple organ systems. Gastrointestinal and renal dysfunction, neurological disorders, skin lesions, vascular damage, immune system dysfunction, congenital abnormalities, and cancer are among the major complications associated with heavy metal toxicity (Balali-Mood et al., 2021; Cobbina et al., 2015; Costa, 2019; Fernandes Azevedo et al., 2012). Heavy metals may promote tumor development through several mechanisms, including damage to cellular organelles and other cellular components, disruption of metabolic enzymes involved in detoxification processes, induction of redox imbalance and uncontrolled oxidation, dysregulation of the cell cycle and cell growth, impairment of DNA repair pathways leading to DNA damage, and modulation of apoptosis and autophagy (Tchounwou et al., 2012). Nevertheless, several metals, including copper (Cu), iron (Fe), selenium (Se), strontium (Sr), manganese (Mn), zinc (Zn), and molybdenum (Mo), are essential for life at physiological concentrations. These metals play protective roles against processes such as chromosomal damage and oxidative stress. By regulating cellular metabolism as

well as DNA, RNA, and protein synthesis, they may exert preventive effects against cancer development (Khoshakhlagh et al., 2024).

Inhibition of Cancer-Related Enzymes by Heavy Metals

Enzyme inhibition is an important mechanism in the regulation of enzymatic activity. The catalytic activity of an enzyme can be suppressed by an inhibitor. Inhibitors are classified into different categories based on their mechanisms of action. Cyanide and many nerve agents are classified as irreversible inhibitors, or catalytic poisons, because they completely abolish enzymatic function (Pearce et al., 2003). Competitive inhibitors possess structural similarities to the substrate molecule and therefore bind to the active site of the enzyme, preventing substrate binding and subsequent catalysis (Robinson, 2015). In contrast, non-competitive inhibitors interact with regions other than the enzyme's active site. These inhibitors generally bind to sites distant from, or in close proximity to, the active site, resulting in conformational alterations that impair enzymatic activity. Heavy metals typically exert non-competitive inhibitory effects due to their strong affinity for sulfhydryl (-SH) groups present in proteins and enzymes. Consequently, heavy metals are often regarded as enzymatic poisons because of their ability to interfere with normal enzymatic function (Mizrahi & Achituv, 1989; Viarengo, 1985). Binding of heavy metals to functional groups within enzyme molecules induces structural modifications that disrupt catalytic activity and alter metabolic processes. As a result, heavy metal exposure can lead to significant toxic effects at the organismal level through the impairment of essential enzymatic pathways (Ashrafi et al., 2019).

LDH and Heavy Metal Inhibition

Lactate dehydrogenase (LDH) is a key oxidoreductase enzyme that catalyzes the conversion of pyruvate to lactate and plays a crucial role in maintaining the Warburg effect in cancer cells. Overexpression of the LDH-A isoenzyme has been associated with enhanced glycolytic activity, increased lactate production, tumor growth, invasion, and metastatic potential in a wide variety of malignancies. Consequently, inhibition of LDH activity has emerged as an important therapeutic strategy for targeting cancer metabolism. Heavy metals can interact with functional groups within proteins, alter the three-dimensional structure of enzymes, disrupt cofactor binding, and impair enzymatic activity through oxidative stress-mediated mechanisms. As a result, LDH is considered one of the metabolic enzymes susceptible to heavy metal-induced inhibition and functional dysregulation (Pan et al., 2025). Heavy metal-mediated inhibition of LDH may occur through both direct and indirect mechanisms. Direct inhibition involves the interaction of metal ions with sulfhydryl, carboxyl, or imidazole groups located within or near the catalytic regions of the enzyme, leading to conformational changes and reduced catalytic efficiency. Indirect inhibition is primarily associated with the generation of reactive oxygen species (ROS),

oxidation of critical amino acid residues, and disruption of cellular redox homeostasis. These alterations may ultimately impair LDH activity and interfere with cellular energy metabolism. Since LDH is a central regulator of glycolytic flux, changes in its activity can significantly affect cancer cell survival and proliferation (Pan et al., 2025). Recent studies have highlighted the importance of heavy metal-induced modulation of LDH activity not only in toxicology but also in cancer biology. Understanding the molecular interactions between LDH and heavy metals may contribute to the development of novel metabolic strategies targeting tumor-associated energy pathways. Therefore, investigation of the inhibitory effects of individual heavy metals on LDH represents an important area of research for both environmental toxicology and cancer therapeutics (Pan et al., 2025).

Effects of Zinc (Zn^{2+}) on LDH Activity

Zinc is one of the essential trace elements involved in maintaining the structural stability of numerous enzymes under physiological conditions. However, at elevated concentrations, Zn^{2+} ions may suppress the activity of various metabolic enzymes. Studies have demonstrated that zinc can bind to glycolytic enzymes such as glyceraldehyde-3-phosphate dehydrogenase (GAPDH), leading to a loss of enzymatic activity, and may also reduce intracellular lactate dehydrogenase (LDH) activity. Increased intracellular Zn^{2+} concentrations, particularly in tumor cells, have been reported to suppress glycolytic flux and decrease ATP production, an effect that has been associated with LDH inhibition. Therefore, zinc is considered a potential metabolic regulator capable of suppressing cancer metabolism (Meng et al., 2022).

The Relationship of Magnesium (Mg^{2+}) with LDH

Magnesium is not traditionally classified as a heavy metal; however, it is one of the most important regulators of cellular energy metabolism. The biologically active form of ATP exists as the Mg-ATP complex, and the activity of numerous metabolic enzymes is dependent on magnesium. Several studies have reported significant associations between serum magnesium levels and LDH levels in cancer patients. Low magnesium concentrations have been linked to increased oxidative stress and mitochondrial dysfunction, both of which may contribute to alterations in LDH activity (Udristioiu & Cojocar, 2021; Mazur et al., 2007). Indeed, the literature indicates that intracellular magnesium deficiency disrupts mitochondrial membrane potential, suppresses cellular respiration, and promotes a compensatory shift toward anaerobic glycolysis. This accelerated glycolytic process, driven by mitochondrial dysfunction, increases the transcriptional regulation of the LDH enzyme complex responsible for catalyzing the pyruvate-to-lactate conversion, resulting in significant fluctuations in serum and tissue LDH levels. These findings provide a biochemical explanation for the observed

correlation between magnesium status and LDH levels in the monitoring of malignancies (Leidi et al., 2011).

The Inhibitory Effect of Copper (Cu^{2+}) on LDH Activity

Copper (Cu^{2+}) is an essential trace metal required for numerous biological processes; however, at elevated concentrations, it can act as a potent modulator of enzymatic activity. Among heavy metals, the inhibitory effects of copper on enzymes represent one of the most extensively characterized mechanisms of metal-induced toxicity. Lactate dehydrogenase (LDH), in particular, has been identified as a highly sensitive target of copper ions. In a study conducted by Pamp et al., Cu^{2+} ions were shown to directly suppress LDH activity, and this inhibitory effect was significantly enhanced in the presence of NADH or NAD^+ . The authors reported that copper increased the accessibility of sulfhydryl ($-\text{SH}$) groups within the enzyme, inducing structural alterations that ultimately resulted in the loss of LDH activity. Furthermore, the study demonstrated that the contribution of reactive oxygen species was limited, indicating that the inhibitory effect was primarily associated with the direct interaction of copper ions with the enzyme molecule itself (Pamp et al., 2005). The inhibitory action of copper on LDH has also been supported by earlier experimental investigations. In an *in vitro* study performed using rat kidney tissue, Dobryszczycka et al. demonstrated that Cu^{2+} ions markedly inhibited LDH activity. The same study also evaluated the effects of Pb^{2+} and Zn^{2+} ions on LDH; however, the inhibitory potency of copper was found to be substantially greater than that of either lead or zinc. The authors reported that Pb^{2+} exhibited approximately 100-fold lower inhibitory capacity, whereas Zn^{2+} displayed nearly 400-fold lower inhibitory activity compared with Cu^{2+} . These findings indicate that copper is among the most effective metal inhibitors of LDH identified to date (Dobryszczycka et al., 1981). From the perspective of cancer metabolism, LDH plays a central role in maintaining the Warburg effect, primarily through the activity of the LDH-A isoform. Elevated LDH activity in cancer cells promotes sustained glycolytic flux and increased lactate production, thereby supporting tumor growth and progression. Recent studies have highlighted LDH as an attractive metabolic target for cancer therapy. In this context, copper-based nanoparticles and copper-containing compounds have been proposed as potential modulators of LDH activity capable of disrupting tumor energy metabolism. Therefore, the inhibitory effects of Cu^{2+} on LDH are of considerable interest not only in toxicological research but also in the development of novel therapeutic strategies aimed at targeting cancer-associated metabolic pathways (Taha et al., 2026).

Glucose-6-Phosphate Dehydrogenase (G6PD) and Heavy Metal Inhibition

Glucose-6-phosphate dehydrogenase (G6PD) is the first and rate-limiting enzyme of the pentose phosphate pathway (PPP) and is responsible for the production of NADPH within cells. Since NADPH is essential for maintaining reduced glutathione levels, supporting lipid biosynthesis, and sustaining cellular defense mechanisms against reactive oxygen species (ROS), G6PD plays a central role in the maintenance of cellular redox homeostasis. Increased G6PD expression has been observed in cancer cells as a consequence of enhanced proliferation and metabolic reprogramming, contributing to tumor growth and survival. Therefore, G6PD has emerged as an important molecular target in cancer metabolism in recent years (Jiang et al., 2014; Sun et al., 2024). Studies investigating the effects of heavy metals on G6PD have demonstrated that numerous metal ions can suppress the catalytic activity of the enzyme through either direct or indirect mechanisms. Kinetic analyses involving Cu^{2+} , Cd^{2+} , Zn^{2+} , and Al^{3+} ions have shown that these metals inhibit G6PD activity and alter the substrate-binding properties of the enzyme. In a recent study by Sun et al., Cu^{2+} and Cd^{2+} ions significantly reduced G6PD activity and decreased the catalytic efficiency of the enzyme. The authors suggested that this effect may result from interactions between metal ions and amino acid residues located near the active site of G6PD (Sun et al., 2024). Similarly, in an inhibition kinetics study conducted by Kırıcı et al., the effects of various heavy metal ions on G6PD were evaluated using molecular modeling approaches. The study revealed that Cu^{2+} , Cd^{2+} , and Zn^{2+} ions exhibit high binding affinity toward the enzyme and may induce activity loss by disrupting the native conformation of G6PD. Among the metals examined, copper and cadmium were reported to exert stronger inhibitory effects than the other metal ions investigated (Kırıcı et al., 2024). The effects of heavy metals on G6PD are not restricted to direct enzyme inhibition. Metals such as cadmium, lead, arsenic, and mercury can induce oxidative stress, deplete intracellular glutathione levels, and disrupt cellular redox balance. Because G6PD is essential for maintaining antioxidant capacity, exposure to heavy metals may result in significant alterations in both the expression and activity of the enzyme. Cadmium exposure, in particular, has been shown to modify G6PD expression, and this effect has been associated with glutathione depletion and oxidative damage (Xu et al., 2003). From the perspective of cancer biology, inhibition of G6PD may increase cellular susceptibility to oxidative stress by reducing NADPH production. Although heavy metal-induced suppression of G6PD activity could theoretically impair the metabolic adaptation of tumor cells, the high toxicity of these metals precludes their therapeutic use. Nevertheless, understanding the molecular mechanisms through which heavy metals affect G6PD may provide valuable insights for the development of novel inhibitors targeting cancer metabolism (Cheng et al., 2025; Sun et al., 2024).

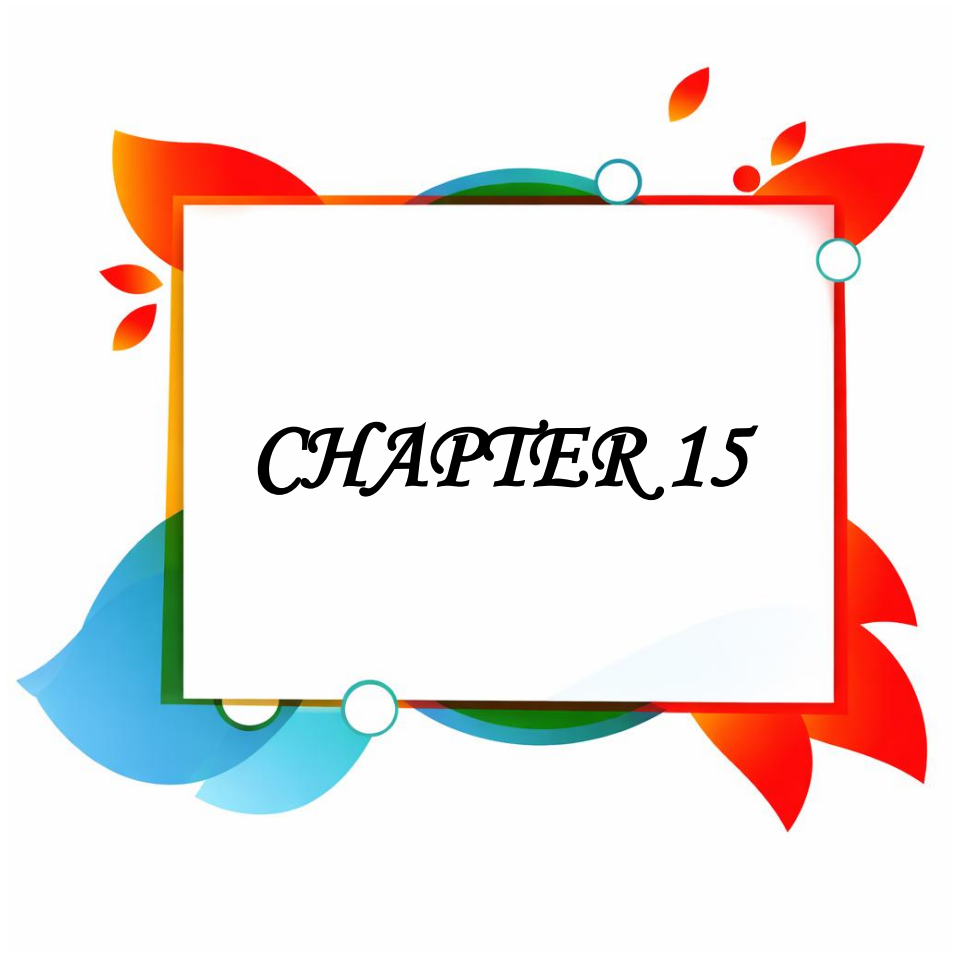
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Physiological Foundations and Systemic Implications of Microbiota–Circadian Rhythm Interactions

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1. Introduction

To adapt to environmental changes, living organisms possess a range of biological timing systems. The most specific example of these systems is the circadian rhythm, which regulates the organism's physiological processes in 24-hour cycles [63,75]. The primary targets of circadian control include the sleep–wake cycle, temperature regulation, hormonal secretions, metabolism, and immune responses [54,66].

For years, it was thought that circadian rhythms were solely driven by the host's genetic and neuroendocrine mechanisms. However, recent studies have shown that the millions of microorganisms living alongside humans are an integral part of this circadian system [77,78]. Particularly, the gut microbiota not only serve as metabolic partners but also actively modulate host physiological rhythms.

Microbiota in the gut exhibit structural and functional changes synchronized with feeding time, light–dark cycles, and hormonal cues [83,77]. Disruption of these rhythmic changes in the microbiota has been associated with the pathophysiology of numerous diseases, including metabolic syndrome, diabetes, obesity, inflammatory bowel diseases, and neuropsychiatric disorders [64,82].

The physiological relationship between the microbiota and circadian rhythm is bidirectional. In this relationship, the host's biological clock shapes the composition of the gut microbiota, while microbiota-derived metabolites influence the expression of clock genes, contributing to the regulation of peripheral circadian clocks [77,49]. Ultimately, this bidirectional communication enhances the organism's energy homeostasis and adaptive capacity.

2. Physiological and Molecular Foundations Of The Circadian Rhythm

2.1. Central Biological Clock: Suprachiasmatic Nucleus (SCN)

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In mammals, the suprachiasmatic nucleus (SCN) is considered the primary regulator of circadian rhythm and the central biological clock, located in the anterior hypothalamus just above the optic chiasm [1,3]. Comprising approximately 20,000 neurons at the center of the organism's endogenous timing system, the SCN synchronizes biological clocks in peripheral tissues [3,4]. The main function of the SCN, which detects environmental time cues (zeitgebers) such as the light–dark cycle, is to align all physiological processes with a 24-hour rhythm [3,5].

The SCN receives light information via retinal signals. This transmission is independent of the classical visual pathway and occurs through the retinohypothalamic tract (RHT) [3]. Intrinsically photosensitive retinal ganglion cells (ipRGCs) within this tract, which contain the photopigment melanopsin and are sensitive to blue light, convey light information to the SCN [3]. This light input plays a critical role in adjusting the circadian phase [3,4], enabling the circadian rhythm to respond to environmental light independently of visual perception [4].

At the molecular level, SCN neurons rely on transcription–translation feedback loops for circadian rhythm generation [4]. CLOCK–BMAL1 heterodimers activate the expression of PER (Period) and CRY (Cryptochrome) genes, forming the molecular basis of the circadian clock [4]. Once PER and CRY proteins accumulate in the cytoplasm and reach a threshold, they return to the nucleus to inhibit the CLOCK–BMAL1 complex, creating a ~24-hour molecular oscillation [4]. The rhythmic activity of SCN neurons manifests as increased firing frequency during the day and decreased firing at night [1,3].

The SCN not only generates intrinsic rhythms but also functions as the master timer for the entire organism via autonomic, neuroendocrine, and behavioral pathways [3,5]. Signals from the SCN regulate melatonin secretion through the pineal gland [12]. Melatonin, which rises during the dark phase of the circadian cycle, conveys “night” information to tissues [12,13]. Its critical role serves as a hormonal bridge between central and peripheral clocks [5,12].

Recent studies have shown that the SCN interacts not only with light but also with metabolic and microbial signals [9,29]. Short-chain fatty acids and tryptophan metabolites derived from the gut microbiota can modulate peripheral clock genes, contributing to circadian alignment between the SCN and peripheral oscillators [9,39]. These findings suggest that the central clock functions as a dynamic regulator interacting with multiple signaling networks rather than an absolute master controller [29,33].

SCN dysfunction leads to disturbances in sleep–wake cycles, hormonal secretion patterns, and metabolic homeostasis [5,30]. Experimental SCN lesions have completely abolished circadian behaviors [1,3], underscoring its indispensable role in circadian regulation [1].

2.2. Peripheral Clocks and Tissue-Specific Rhythms

While circadian regulation is centered on the SCN, it is not the sole regulator [3,5]. Numerous peripheral tissues—including the gut, liver, pancreas, adipose tissue, skeletal muscle, and immune cells—possess autonomously operating clocks [5,31]. These are referred to as peripheral clocks and, at the molecular level, utilize clock genes similar to those in the SCN [4,5].

The primary molecular oscillators of peripheral clocks are CLOCK, BMAL1, PER, and CRY genes [4]. However, their phase and amplitude differ from the central clock due to responsiveness to feeding time, hormonal cues, and metabolic inputs rather than light [31,38]. This feature renders peripheral clocks physiologically sensitive to microbial influences [29,41].

The liver is one of the best-characterized peripheral tissues [5,38]. Hepatic circadian rhythms regulate processes such as gluconeogenesis, glycogen metabolism, lipid synthesis, and bile acid production [5,38]. Changes in feeding time can shift liver clock phases independently of the SCN [31], resulting in central–peripheral desynchronization [30,41].

The gut holds a unique position within circadian rhythms [8,29], as enterocyte proliferation, gut motility, mucus secretion, and epithelial permeability vary throughout the day [8,37]. These variations are directly influenced by the gut microbiota [8,29]. Consequently, distinct bacterial populations dominate in different day–night phases, modulating clock gene expression in segment-specific epithelia [8,39].

Endocrine functions of the pancreas are also under circadian control [5,10]. Insulin secretion, beta-cell sensitivity, and glucose tolerance fluctuate across the day [10,30]. Disruption of peripheral clock genes is linked to beta-cell dysfunction and diabetes, highlighting the circadian rhythm–metabolic disease–microbiota triangle [11,29].

The key feature differentiating peripheral from central clocks is their dissociable nature [30]. In cases of irregular sleep, inconsistent eating patterns, night eating, shift work, or jet lag, the SCN remains aligned with the light–dark cycle, while peripheral clocks adjust to food intake [18,30]. This results in “internal desynchronization,” a core factor in metabolic disorders [30,38].

Microbiota emerge as critical regulators in this context [29,33]. Microbial metabolites such as short-chain fatty acids, secondary bile acids, and indole derivatives directly modulate peripheral clock gene expression [9,39], establishing microbiota as active timekeeping components rather than passive outcomes [29,41].

Thus, while coordinated by the central clock, peripheral clocks remain highly sensitive to metabolic and microbial signals [5,29], forming the physiological

basis for microbiota–circadian interactions primarily in peripheral tissues [29,41].

2.3. Feeding Time and Microbiota-Mediated Circadian Synchronization

Feeding is one of the most potent zeitgebers for peripheral clocks. While the SCN primarily responds to the light–dark cycle, peripheral tissues are highly sensitive to feeding timing, frequency, and composition, making feeding schedules crucial for maintaining circadian integrity [2,3].

The stomach and intestines, which first respond to nutrient intake, play central roles in circadian synchronization. Feeding habits at different times affect gut motility, digestive enzyme secretion, and epithelial permeability, directly influencing gut microbiota composition and metabolic activity [8,29].

Animal studies have shown that regular feeding schedules induce pronounced diurnal fluctuations in gut microbiota [29,39]. Microbial taxa exhibit distinct dominance during day and night, demonstrating that the microbiota is a dynamic structure whose activity is circadian-dependent rather than a passive ecosystem [29,33]. Disruption of feeding times abolishes microbial rhythmicity and fosters dysbiosis [29,41].

Microbiota modulate circadian clocks via metabolites produced in response to host feeding. Short-chain fatty acids like acetate, propionate, and butyrate influence clock gene expression in intestinal epithelial cells and hepatocytes, adjusting peripheral clock phases [9,39]. This indicates that microbial metabolites function not only as energy substrates but also as chronometabolites [9,29] (Figure 1).

Multi-omics integration of circadian—microbiota crosstalk

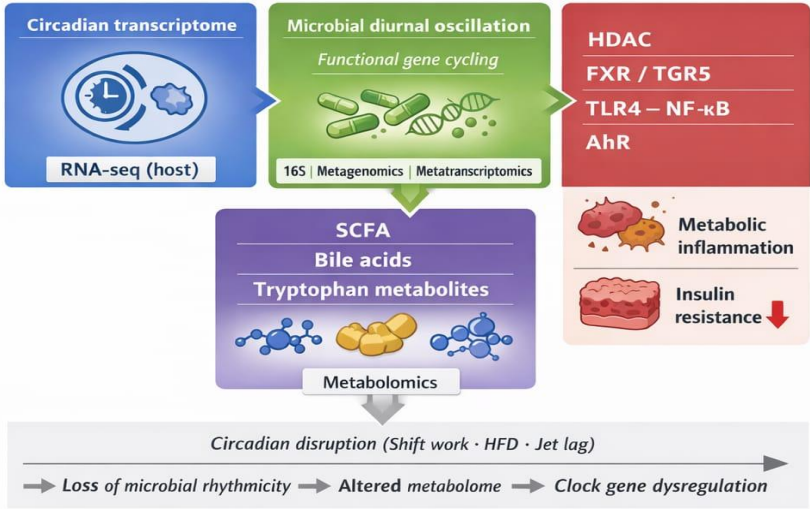


Figure 1. Multi-Omics Conceptual Framework

Time-restricted feeding, which limits food intake to specific hours of the day, aims to align feeding with circadian rhythms regardless of total caloric intake. Experimental studies demonstrate that this approach restores microbiota rhythmicity, reduces metabolic inflammation, and normalizes peripheral clock gene expression [29,38], with effects largely mediated by the microbiota [29,41].

Late-night eating or irregular meal timing disrupts synchronization between central and peripheral clocks [30,38]. While SCN clocks follow the light–dark cycle, peripheral gut and liver clocks reset according to feeding time [3,31]. This internal desynchronization is associated with glucose intolerance, insulin resistance, and lipid metabolism disorders [10,30]. Microbiota both contribute to and are affected by this dysregulation [29,41].

Nutrient composition also matters. High-fat diets alter microbiota composition, disrupting circadian gene expression [29,41]. Diets rich in saturated fats increase microbiota-derived inflammatory signaling and suppress clock genes [29,33], whereas high-fiber diets enhance short-chain fatty acid production and support circadian synchronization [9,39] (Table 1).

Overall, feeding time sits at the center of microbiota–circadian interactions. Microbiota actively synchronize peripheral clocks through rhythmic responses

and metabolite production [29,39], emphasizing the physiological and clinical importance of chronobiology-based nutritional strategies [2,29].

Table 1. Microbial Metabolites and Circadian

EffectsMetabolite	Mechanism	Circadian Impact	Systemic Effect
SCFA (Butyrate)	HDAC inhibition	Modulates BMAL1	Improves insulin sensitivity
Bile Acids	FXR/TGR5 activation	Regulates metabolic genes	Lipid balance
Tryptophan Metabolites	AhR signaling	Immune clock modulation	Anti-inflammatory
LPS	TLR4 activation	Suppresses clock genes	Chronic inflammation

2.4. Physiological Consequences of Circadian Rhythm Disruption

The circadian rhythm is the primary regulatory system aligning the internal environment with temporal changes in the external world. Disruption of this rhythm affects not only sleep–wake cycles but also metabolism, endocrine balance, immune function, and neurological processes [1,2]. Modern lifestyle factors—shift work, irregular eating, and artificial light exposure—increase the prevalence of circadian disruption [18,30].

One of the earliest effects of circadian disruption is impaired metabolic homeostasis. Glucose tolerance, insulin sensitivity, and energy expenditure exhibit daily rhythmic fluctuations [10,30]. Disruption, particularly with nocturnal feeding, results in marked postprandial glucose elevations, reflecting peripheral clocks desynchronized from the SCN [30,38].

The endocrine system is another directly affected target. Hormones such as cortisol, melatonin, growth hormone, and leptin follow circadian secretion patterns [5,12]. Rhythm disruption flattens cortisol and suppresses melatonin secretion [12,13], negatively affecting sleep quality, epithelial barrier integrity, and microbiota balance [12,29].

Immune functions are temporally organized. Leukocyte circulation, cytokine secretion, and inflammatory responses fluctuate throughout the day [5,30]. Circadian disruption increases proinflammatory cytokines, promoting low-grade chronic inflammation [29,33] and altering microbiota composition, facilitating dysbiosis [29,41].

The gastrointestinal system is particularly sensitive to circadian disruption due to rhythmic regulation of motility, mucus secretion, and epithelial regeneration [8,29]. Disruption increases gut permeability, predisposing to “leaky gut” and facilitating systemic inflammation [8,39; 29,33].

Neurophysiologically, circadian disruption correlates with impaired cognitive performance, attention deficits, and mood changes [1,30]. Sleep disturbances

disrupt REM and non-REM balance, negatively impacting learning and memory [1,3]. Microbiota-derived neuroactive metabolites are believed to contribute to these processes [29,33].

Long-term circadian disruption is considered a risk factor for obesity, diabetes, cardiovascular, and neurodegenerative diseases [10,30]. Experimental studies show that clock gene mutations shorten lifespan and accelerate aging [4,30], highlighting circadian rhythms as fundamental physiological protective mechanisms [1,4].

Thus, circadian disruption is a systemic pathophysiological condition affecting multiple organ systems. The bidirectional microbiota–circadian interaction plays a critical role in both disease onset and progression [29,41]. Maintaining circadian rhythm is therefore vital for physiological balance and disease prevention [2,29].

3. Human Microbiota and Circadian Rhythm: Structural, Metabolic, and Molecular Interactions

The human gastrointestinal tract hosts approximately 10^{13} – 10^{14} microorganisms, collectively termed the “microbiota” [1,2]. This community, with genetic diversity far exceeding that of the human genome, is now recognized as an integral component of host physiology [3]. The microbiota is dynamic, actively influencing metabolic, immune, and endocrine processes [3,4].

In the gut, the phyla Firmicutes and Bacteroidetes dominate, while Actinobacteria, Proteobacteria, and Verrucomicrobia are present at lower levels [1,5]. Gut composition is influenced by age, genetics, diet, hormonal status, and circadian rhythms [5,6]. Microbial density is not uniform along the gut; it is relatively low in the small intestine and forms a high-density anaerobic ecosystem in the colon [1]. These regional differences are closely linked to the timing of local metabolic functions [6].

3.1. Metabolic Functions of the Microbiota

The microbiota ferments complex carbohydrates indigestible by the host to produce short-chain fatty acids (SCFAs) [7]. Major SCFAs—acetate, propionate, and butyrate—serve not only as energy sources for colonocytes but also exert regulatory effects on systemic metabolism [7,8].

SCFAs regulate glucose homeostasis, lipid metabolism, insulin sensitivity, and energy expenditure [8]. Butyrate, the primary energy substrate for colonocytes, is critical for maintaining intestinal epithelial barrier integrity [7], demonstrating the microbiota’s structural as well as metabolic significance [4].

3.2. Microbiota and Endocrine Physiology

The microbiota and the endocrine system communicate bidirectionally and dynamically [4,9]. Hormones related to appetite and energy balance, such as glucagon-like peptide-1 (GLP-1), peptide YY (PYY), and ghrelin, are influenced by gut bacteria, affecting energy intake and glycemic control [9,10].

Additionally, the microbiota modulates bile acid transformations, influencing metabolic gene expression via nuclear receptor FXR and membrane-bound TGR5, serving as physiological bridges integrating hepatic and intestinal metabolic clocks [6,11].

3.3. Rhythmic Behavior of the Microbiota

Recent studies reveal that the microbiota is not static but undergoes circadian fluctuations [6,12]. Bacterial diversity, gene expression, and metabolite production oscillate in synchrony with the circadian clock [12], shaped particularly by feeding time and hormonal signals [6].

Daily variations in melatonin and cortisol indirectly influence microbial diversity [13], making the microbiota an integrated biological component rather than a passive circadian target [6,12].

Thus, the microbiota functions as an active modulator of molecular circadian regulation, not merely a physiological actor [6,12]. The underlying mechanisms are discussed in the following sections.

3.4. Molecular Mechanisms of Microbiota–Circadian Interactions

The microbiota–host circadian relationship is multilayered, involving numerous molecular interactions [6,12], including metabolic products, immune responses, epigenetic regulation, and direct modulation of clock genes [6,14]. Evidence indicates that the microbiota regulates the phase, amplitude, and stability of peripheral clocks [12].

3.4.1. Direct Clock Gene Interactions

The molecular basis of circadian rhythm involves CLOCK, BMAL1, PER, and CRY genes, regulated not only by endogenous oscillations but also by metabolic and microbial signals [15]. Germ-free animal studies show disrupted peripheral clock gene expression, with reduced BMAL1 and PER2 amplitude, suggesting that microbiota are essential for peripheral clock stability [12].

3.4.2. SCFAs and Epigenetic Regulation

SCFAs regulate gene expression via G-protein-coupled receptors GPR41 and GPR43 and through histone deacetylase inhibition [7,14]. Butyrate's HDAC inhibition plays a key role in epigenetic control of clock genes [14]. Circadian fluctuations in SCFA levels suggest temporal modulation of CLOCK–BMAL1 activity in peripheral tissues, positioning SCFAs as chronometabolites linking feeding and circadian rhythm [9,29,14].

3.4.3. Bile Acids and Nuclear Receptor Signaling

Primary bile acids synthesized in the liver are converted to secondary bile acids by the microbiota, affecting metabolic and circadian gene expression via FXR and TGR5 [11,16]. Diurnal bile acid fluctuations are crucial for synchronizing liver and gut clocks, with disruptions predisposing to metabolic disease [11,16].

3.4.4. Toll-like Receptors and the Immune Clock

Toll-like receptors (TLRs) in intestinal epithelium and immune cells recognize microbial components, initiating immune responses [17]. TLR expression shows circadian oscillation [18]; for example, diurnal TLR4 fluctuations explain time-dependent lipopolysaccharide responses, forming the molecular basis of the microbiota–immune–circadian axis [18].

3.4.5. Epigenetic Mechanisms

The microbiota influences host gene expression via DNA methylation, histone modifications, and microRNA regulation [14]. These changes contribute to long-term regulation of clock genes [15]. Epigenetic modifications dependent on feeding time and microbiota composition determine peripheral clocks' capacity to adapt to metabolic cues [6].

3.4.6. Indirect Communication Between Central and Peripheral Clocks

Although the microbiota does not directly target the SCN, it communicates with the central clock indirectly through peripheral clocks [6]. Metabolic and hormonal feedback mechanisms maintain synchronization between central and peripheral clocks [6,12].

4. Bidirectional Physiological Interaction Between Microbiota and Circadian Rhythm

The bidirectional relationship between gut microbiota and the circadian rhythm does not follow a unidirectional regulatory mechanism; rather, it represents a dynamic and reciprocal physiological network. Experimental and clinical studies have demonstrated that microbiota diversity fluctuates throughout the day, and these fluctuations are shaped primarily by the host's circadian timing system, with feeding time being a major determinant [8,29].

Within the host organism, central and peripheral clocks influence the daily rhythm of the intestinal ecosystem through several physiological processes, including timing of nutrient intake, intestinal motility, mucus secretion, bile acid release, and epithelial cell turnover [3,5]. These processes provide temporally dynamic environmental conditions in the gut lumen, which in turn regulate microbial diversity and functionality [8,29]. Specifically, the rhythmic activity of CLOCK and BMAL1 genes expressed in intestinal epithelial cells has been shown to temporally control antimicrobial peptide secretion and barrier integrity [4,14].

The influence of the microbiota on the host circadian rhythm largely occurs via microbial metabolites. Short-chain fatty acids (SCFAs) such as acetate, propionate, and butyrate, as well as secondary bile acids and tryptophan metabolites, regulate the expression of clock genes in peripheral tissues, thereby modulating the timing of metabolic and immune processes [9,39]. A prominent example of the microbiota's direct molecular effect on the circadian system is butyrate acting as a histone deacetylase inhibitor to epigenetically regulate clock gene expression [14].

From a metabolic homeostasis perspective, this bidirectional interaction is critical. Processes such as glucose metabolism, insulin sensitivity, and lipid synthesis are simultaneously regulated by both circadian clock genes and microbiota activity [10,29]. Disruption in this coordination, whether through altered feeding schedules, microbiota dysbiosis, or circadian misalignment, can lead to metabolic disorders, including obesity, insulin resistance, and type 2 diabetes [10,30].

Additionally, the microbiota–circadian interaction extends to immune regulation. SCFAs and other microbial metabolites modulate the rhythmic expression of immune-related genes and the secretion of cytokines, thereby influencing the diurnal oscillations of host immune responses [29,33]. This ensures that the immune system's activity is synchronized with the organism's metabolic and behavioral cycles, optimizing defense mechanisms and maintaining tissue homeostasis.

The influence of microbiota on circadian rhythm is not limited to peripheral tissues. Recent evidence suggests that microbial metabolites can signal indirectly to the central clock in the suprachiasmatic nucleus (SCN) via hormonal and metabolic feedback loops, integrating systemic cues to fine-tune circadian timing across the organism [6,12]. Thus, the gut microbiota acts as both a mediator and a participant in the host's temporal physiology, rather than merely a passive bystander.

In summary, the interplay between microbiota and circadian rhythm constitutes a tightly coordinated network in which microbial metabolites, clock genes, and host physiological processes influence each other. This bidirectional

communication underpins metabolic, immune, and endocrine homeostasis and highlights the critical role of temporal regulation in maintaining health. Disruptions in this network contribute to the pathophysiology of metabolic, inflammatory, and neuropsychiatric disorders, emphasizing the importance of maintaining circadian integrity for overall physiological balance [2,29,41] (Table 2).

Table 2. Circadian Disruption and Microbiota Changes

Disruption Type	Microbiota Change	Metabolic Impact	Clinical Outcome
Jet Lag	Reduced diversity	Glucose intolerance	Obesity risk
High-Fat Diet	Firmicutes increase	Lipid accumulation	Metabolic syndrome
Shift Work	Reduced SCFA	Insulin resistance	T2DM risk

5. Microbiota–Circadian Rhythm Interactions and Their Impact on Metabolic Physiology

5.1. Circadian Organization of Energy Homeostasis

Energy homeostasis, the balance between energy intake, storage, and expenditure, is tightly regulated by circadian rhythms. Central clocks and peripheral tissue clock genes coordinate energy utilization strategies across the day to optimize metabolic efficiency. Clock genes in the liver, skeletal muscle, and adipose tissue drive rhythmic fluctuations in mitochondrial oxidative capacity, substrate preference, and basal metabolic rate [8,34,40].

The gut microbiota contributes significantly to the circadian regulation of energy homeostasis. Fermentation of dietary fibers by microbial communities produces short-chain fatty acids (SCFAs), which serve as local energy sources and signaling molecules affecting host metabolism. The diurnal oscillation of microbial metabolite production indicates an active role for the microbiota in the temporal coordination of energy balance [46,47,48].

5.2. Glucose Metabolism and Insulin Sensitivity

Glucose metabolism and insulin sensitivity are central metabolic processes under circadian control. Insulin secretion from pancreatic beta cells, peripheral tissue responses, and hepatic gluconeogenesis exhibit diurnal rhythmicity, synchronized with feeding times [1–3,58,59].

The microbiota influences glucose metabolism both directly and indirectly. SCFAs stimulate L-cells in the intestine to release GLP-1 and PYY, supporting insulin secretion and appetite regulation [20,21,79]. Circadian disruption reduces the amplitude of these hormonal responses, contributing to insulin resistance [6,56]. Dysbiosis also increases intestinal permeability, promoting low-grade inflammation and impairing insulin signaling [22,31].

5.3. Lipid Metabolism and Adipose Tissue Physiology

Lipid metabolism, including fatty acid synthesis, lipolysis, and lipoprotein turnover, is regulated by circadian clock genes [1–3,34]. Genes involved in hepatic lipogenesis and enzymes regulating adipose tissue lipolysis display diurnal expression patterns, maintaining the physiological balance between energy storage and mobilization [4,5].

The microbiota modulates bile acid metabolism, affecting lipid absorption and cholesterol homeostasis [26,44,65]. Secondary bile acids signal through nuclear and membrane receptors, including FXR and TGR5, to regulate genes involved in lipid metabolism [46,109]. Disruptions in microbiota-derived bile acid signaling can cause circadian misalignment in lipid metabolism. In adipose tissue, microbiota–circadian interactions influence adipokine secretion patterns, shaping systemic metabolic responses [11,12,76].

5.4. Microbiota in Obesity and Metabolic Syndrome

Obesity and metabolic syndrome are closely associated with both circadian disruption and microbiota imbalance [1–3,80,81]. Shift work, chronic sleep deprivation, and irregular eating patterns reduce microbial diversity and exacerbate metabolic risk factors [4,5,55].

Dysbiosis in these conditions promotes increased energy extraction from diet, enhances inflammatory responses, and perturbs hormonal signaling [6,7,22,64]. Experimental models show that transferring microbiota from circadian-disrupted animals to healthy recipients can induce obesity-like phenotypes, highlighting the role of microbiota–circadian interactions in metabolic disease pathogenesis [9,10,64].

6. Microbiota, Circadian Rhythm, and the Immune System

6.1. Circadian Organization of the Immune System

The immune system is a dynamic physiological network regulated by circadian rhythms. Both innate and adaptive immune responses, including leukocyte trafficking, cytokine release, and antigen presentation, show time-of-day–dependent variations [1,2,24,28].

The central clock in the suprachiasmatic nucleus (SCN), through autonomic and hormonal signals, modulates immune cell activity [1,2,32]. Cortisol peaks in the morning suppress proinflammatory cytokine production, while nighttime melatonin enhances T-cell proliferation and antibody responses [2,13,19,60].

6.2. Role of Microbiota in Immune Physiology

The microbiota educates and regulates immune development and function. Microbial antigens and metabolites, such as acetate, propionate, and butyrate, directly influence immune cell differentiation and cytokine production [5,38,68].

Butyrate, in particular, promotes regulatory T cell (Treg) development and suppresses inflammatory responses [38,68].

Diurnal variations in microbiota composition and activity affect immune response timing, with gut barrier function, mucus secretion, and antimicrobial peptide production showing circadian patterns [7,8,73,74]. Microbiota-derived signals can also activate Toll-like receptors, altering peripheral clock gene expression in immune cells [8,10,66].

6.3. Inflammation, Dysbiosis, and Circadian Disruption

Circadian disruption is linked to chronic inflammation. Factors such as sleep deprivation, shift work, and irregular eating patterns disturb central and peripheral clocks, increasing proinflammatory cytokines (TNF- α , IL-6, IL-1 β) [2,4,42].

Dysbiosis resulting from circadian misalignment activates inflammatory signaling and suppresses core clock gene expression [7,52]. CLOCK and BMAL1 interact with NF- κ B pathways, explaining the molecular basis of increased inflammation during circadian disruption [52,57].

6.4. Autoimmune and Inflammatory Diseases

Microbiota and circadian misalignment play increasingly recognized roles in autoimmune and chronic inflammatory diseases, including rheumatoid arthritis, inflammatory bowel disease, multiple sclerosis, and systemic lupus erythematosus [2,5,32,43].

Symptoms often exhibit diurnal patterns (e.g., morning stiffness in rheumatoid arthritis), indicating circadian regulation of immune responses. Mis-timed microbiota signals can compromise immune tolerance, exacerbating autoimmunity [3,8]. Chronotherapy and microbiota-targeted interventions are emerging as physiologically informed therapeutic strategies [69,70,71].

7. Nervous System, Sleep, and Microbiota

The nervous system orchestrates circadian rhythm regulation and interacts bidirectionally with the microbiota to maintain sleep–wake cycle integrity [1–3,23,27].

The gut–brain axis, comprising the central, autonomic, and enteric nervous systems, transmits microbial signals that influence sleep architecture, melatonin secretion, and cortical activity patterns [23,27,67]. Microbiota-derived neuroactive metabolites, including GABA, serotonin precursors, and SCFAs, modulate central nervous system function and sleep quality [72,27,67].

Peripheral serotonin synthesis in the gut, synchronized with circadian clocks, provides substrates for melatonin production [9,10,60]. The hypothalamic–pituitary–adrenal (HPA) axis links stress responses to circadian rhythm;

microbiota dysbiosis can hyperactivate the HPA axis, flatten cortisol rhythms, disrupt sleep, and trigger neuroinflammation [35,53,129].

Vagus nerve signaling mediates microbiota–brain communication, modulating parasympathetic tone and sleep initiation [16,17,18].

8. Clinical Applications and Therapeutic Approaches

Elucidating the physiological interactions between microbiota and circadian rhythms enables novel, integrative therapeutic strategies [1–3,25]. Chronobiology emphasizes not just ‘‘what’’ to treat but ‘‘when’’ to treat [4,5,69].

Time-restricted feeding (TRF) regulates microbiota rhythmicity, improving metabolic and inflammatory parameters [25,41]. Administration of probiotics and prebiotics at specific times enhances peripheral clock gene expression, increasing therapeutic efficacy [45,50,51].

Sleep disturbances, metabolic syndrome, diabetes, and autoimmune diseases may benefit from interventions targeting circadian rhythm alignment alongside pharmacological and lifestyle measures. Light exposure, melatonin supplementation, and stress management indirectly but significantly influence microbiota composition [14–16,61,62]. Personalized microbiota-based treatment protocols are anticipated to enter clinical practice as an innovative strategy for restoring physiological balance [17,18,76] (Table 3).

Table 3. Summary of molecular, microbial, and systemic components involved in circadian–microbiota interactions

Level	Component	Key Elements	Mechanisms / Functions	Physiological / Systemic Impact	References
Host Circadian Clock	Core clock genes	BMAL1, CLOCK, PER, CRY	Transcription-translation feedback loops	Regulates sleep-wake cycles, hormone secretion, metabolism	7, 54, 58
Central Nervous System	SCN & Peripheral clocks	Suprachiasmatic nucleus, liver, gut	Light entrainment, hormonal regulation	Circadian regulation of metabolism, immune function	54, 59, 58
Microbiota	Composition & Diversity	Firmicutes, Bacteroidetes, Proteobacteria	16S rRNA, metagenomics, metatranscriptomics	Modulates energy harvest, SCFA production, bile acid metabolism	36, 38, 46
Microbial Metabolites	SCFA, bile acids, tryptophan derivatives	Butyrate, propionate, indole derivatives	HDAC inhibition, FXR/TGR5, AhR signaling	Epigenetic regulation, inflammation modulation, metabolic homeostasis	5, 38, 46, 65, 68
Immune System	Innate & Adaptive	T cells, macrophages, cytokines	NF-κB, inflammasome activation, Treg modulation	Immune rhythmicity, inflammation, autoimmunity	4, 10, 28, 29, 52
Metabolic Outcomes	Glucose & Lipid metabolism	Insulin sensitivity, weight gain	Time-restricted feeding, circadian misalignment	Obesity, insulin resistance, metabolic syndrome	3, 25, 41, 47, 48

Environmental Inputs	Light, feeding schedule, sleep	Night-time light, irregular feeding	Alters clock gene expression	Dysbiosis, circadian disruption, systemic inflammation	9, 55, 56

9. Future Perspectives and Conclusions

Microbiota–circadian rhythm interactions act as an integrative regulator across multiple physiological systems [1–3,76]. Current evidence highlights their pivotal roles in metabolic, immune, and neurological processes, yet many fundamental questions remain [4,5,58,76].

Future studies should focus on cell type–specific clock gene responses to microbial signals and systematically explore temporal factors in human studies. Multi-omics approaches—including metagenomics, metabolomics, and transcriptomics—will clarify individual differences in microbiota–circadian interactions [8–10,36,48].

Maintaining circadian–microbiota harmony is critical for homeostasis, and disruptions contribute to chronic disease pathogenesis. Rhythm-based therapeutic strategies are poised to become central in preventive and precision medicine [13–15,69,70].

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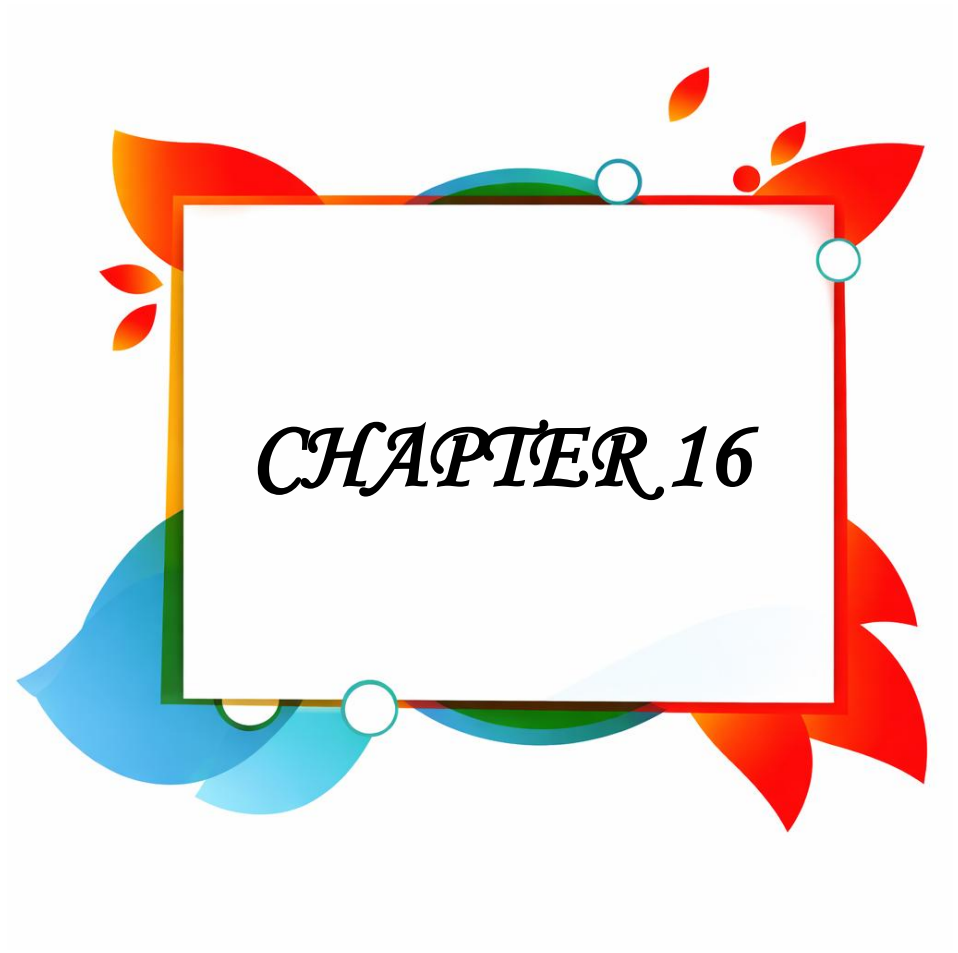
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Use of Social Media in Public Health: Vaccination, Vaccine Hesitancy and Digital Infodemic

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Introduction

Vaccination is one of the most fundamental areas of public health intervention for the prevention of infectious diseases, the reduction of disease burden, and the strengthening of community immunity. Vaccination programs depend not only on the biological effectiveness of vaccines and the infrastructure of service delivery, but also on the level of trust individuals have in health authorities, scientific evidence, and vaccination policies. In recent years, social media has moved beyond being a complementary tool of public health communication and has become a central public sphere in which health information is produced, disseminated, discussed, and reframed. Platforms such as YouTube, Facebook, X/Twitter, Instagram, and TikTok enable health institutions to reach the public rapidly, increase the visibility of vaccination campaigns, share up-to-date information during crises, and monitor public concerns. At the same time, these platforms also function as spaces in which unverified content, misinterpretations of scientific uncertainty, conspiracy narratives, and anti-vaccine discourse can spread rapidly. The COVID-19 pandemic clearly demonstrated that, in addition to vaccine development and implementation, it is also critical to understand how vaccine-related information circulates in society, which sources it draws upon, and within which trust relationships it is interpreted (Larson et al., 2022). Therefore, in the context of vaccination, social media should be regarded as a strategic public health environment that, beyond its communicative function, influences trust, risk perception, information pollution, political polarization, and behavioral decision-making processes (Rodrigues et al., 2024).

In this chapter, the concept of digital infodemic is used to refer to the excessive circulation of accurate, inaccurate, incomplete, distorted, or intentionally misleading health-related information in digital environments, which makes it more difficult for individuals to access reliable information. Misinformation refers to the sharing of inaccurate information without the intent to mislead, whereas disinformation refers to the deliberate production or dissemination of false information. Social listening refers to the systematic monitoring of public

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opinions, concerns, questions, and reactions on social media and online platforms; infoveillance refers to the analysis of this digital information flow for public health surveillance and early warning purposes. These concepts indicate that vaccination communication is not limited to message production, but should also be addressed together with the monitoring of the information ecosystem, the early detection of risks, and the development of targeted communication strategies (Sanchez et al., 2026).

This chapter evaluates the role of social media in public health and vaccination along the axes of communication, trust, risk perception, information pollution, infoveillance, and behavioral decision-making processes. While social media facilitates the dissemination of reliable public health information, it also creates a favorable digital environment for the circulation of misinformation and disinformation. Although social media data provide capacity for monitoring public concerns, social listening, and infoveillance, they also have important limitations related to representativeness, ethics, privacy, and algorithmic bias. Social media-based interventions may offer advantages in terms of broad reach and low cost; however, engagement indicators do not always correspond to actual vaccination behavior. Within this framework, the chapter addresses social media not as a standalone risk domain or an inherently effective solution, but within the broader integration of digital infodemic, vaccine hesitancy, social listening, infoveillance, and public health communication.

Social Media and Public Health Communication

Public health institutions use social media extensively to announce vaccine recommendations, promote vaccination campaigns, address public concerns, increase vaccine confidence, share visual materials such as videos, infographics, and testimonial narratives, and maintain emergency communication during outbreaks. The way in which the COVID-19 vaccination campaign in Israel was framed by the Ministry of Health demonstrates how institutional actors position social media as a strategic tool in crisis communication (Gesser-Edelsburg et al., 2022). The public relations strategies of the Ghana Health Service also show that institutions use social media as a tool for disseminating messages at a mass scale (McCarthy et al., 2023). The “AUBe Vaccinated” campaign in Lebanon provides an example of how social marketing principles can be applied through social media to increase vaccination rates on a university campus (Bardus et al., 2023). A social media campaign implemented in Nigeria likewise shows that digital communication can produce measurable outcomes at the field level (Evans et al., 2023). However, since a substantial proportion of these studies are based on context-specific campaign evaluations, the direct generalizability of their findings to different countries, platforms, and target populations is limited. The individual use of social media by health care workers may also increase trust in science (Jain et al., 2022). Interventions conducted through micro-influencers

may be used as a complementary strategy, particularly in local communities and communities with low levels of trust (Hohmeier et al., 2022).

The turn of institutions and individual actors toward social media is driven by the operational advantages of these platforms, including rapid information dissemination, broad reach, low cost, interactive communication, real-time feedback, and the ability to target specific subgroups. A large-scale field experiment examining the effects of digital public health advertising on COVID-19 vaccine beliefs and outcomes showed that social media advertising generated meaningful but limited effects on beliefs and behavior (Athey et al., 2023). This finding suggests that social media campaigns can be effective, while also demonstrating that reach and engagement indicators should not be interpreted on their own as public health success. From a service theory perspective, patterns of engagement behavior during the COVID-19 vaccination process indicate that public health service communication should be evaluated not only in terms of message content, but also together with service access, trust, and interaction design (Azer & Alexander, 2022).

The opportunities offered by social media are intertwined with a number of structural limitations. Inequalities in access, low trust in institutional accounts, competition with misinformation, difficulties in translating engagement into behavior change, and the lack of transparency in platform algorithms are among the leading limitations. A focus group study on information access among foreign-born individuals in Sweden demonstrates that language, cultural adaptation, and institutional trust issues restrict the reach of social media communication (Soederberg et al., 2024). Thus, social media is not an equitable communication solution on its own, and its effects vary according to platform, country context, target population, vaccine type, and outcome measures (Rodrigues et al., 2024). This heterogeneity provides the basic ground for explaining why the relationship between social media, vaccine hesitancy, and social attitudes produces different results across countries and platforms.

Social Media and Vaccine Hesitancy

Vaccine hesitancy narratives circulating on social media largely cluster around recurring themes. These mainly include safety concerns, side effects, infertility concerns, claims linking vaccines to autism, distrust of pharmaceutical companies, distrust of government, natural immunity narratives, religious or moral concerns, low perceived disease risk, conspiracy theories, and political identity-based polarization. Reviews demonstrating that the alleged relationship between autism spectrum disorder and vaccination lacks a scientific basis indicate that this narrative is persistently reproduced on social media (Gabis et al., 2022). A study examining the relationship between information trust and vaccine hesitancy among middle-aged and older adults in Singapore showed that different typologies of trust shape levels of hesitancy in different ways (Tan et al., 2022).

Sentiment analysis based on Twitter data in Iran revealed that public emotional responses toward vaccines followed a fluctuating pattern over time (Nezhad & Deihimi, 2022). Taken together, these findings indicate that vaccine hesitancy cannot be explained solely by information deficit and should be analyzed together with trust, identity, risk perception, and political context. In the Lebanese context, conspiracy beliefs were shown to reduce vaccination intention, while conspiracy narratives linking 5G technology and COVID-19 were found to draw on erroneous inferences related to insufficient data literacy (Flaherty et al., 2022; Ghaddar et al., 2022). The fact that narratives such as the autism myth, distrust of pharmaceutical companies, and religious concerns contribute to hesitancy even among health care workers demonstrates that hesitancy discourse is not limited to the general public (Njoga et al., 2022; Riad et al., 2022).

Pro-vaccine content that stands in contrast to hesitancy narratives is shaped around messages related to the protection of children and society, emphasis on scientific evidence, endorsement by health care workers, personal narratives, collective responsibility, return to normal life, and disease prevention. The “One Vax Two Lives” social media campaign and research program conducted in North Carolina showed that trust-oriented messages about vaccination during pregnancy can be strengthened through community-based narratives (Marcell et al., 2022). A study examining perceptions, knowledge, and attitudes related to vaccine hesitancy among older Portuguese adults also points to the importance of information sources that enhance trust (Silva et al., 2022). Nevertheless, the impact of pro-vaccine messages is determined not only by scientific accuracy, but also by the extent to which the message aligns with the values, prior experiences, and level of institutional trust of the target population. A study examining community-based determinants of COVID-19 vaccine hesitancy in Austria showed that political orientation and institutional trust strongly explained attitudes (Till & Niederkrotenthaler, 2022). The finding that economic risk framing increased vaccination intention among Republican voters in the United States revealed that political identity interacts with message framing (Zhong & Broniatowski, 2023). Cultural and social attitudes and factors associated with COVID-19 vaccine acceptance among adults across different global contexts also support the need for such contextual interpretation (Begum et al., 2024). Overall, social media studies offer a strong research field in terms of access to large-scale data, real-time observation, and the ability to analyze public discourse. However, findings should be interpreted cautiously in terms of representativeness, measurement validity, and causality. Social media users may not always represent the general population. Therefore, platform interactions should not be interpreted as direct indicators of individual beliefs. Similarly, emotional tone may not directly reflect vaccination intention. Online discourse may also make extreme views appear more visible than they actually are (Faye et al., 2022; Moscardino et al., 2022). For this reason, hesitancy and trust narratives observed on social

media should not be interpreted as direct behavioral indicators, but rather as early signals reflecting societal risk perception, trust problems, and communication gaps. This methodological caution also requires misinformation, disinformation, and infodemic dynamics to be addressed within the same critical framework.

Misinformation, Disinformation, and Infodemic

Vaccine misinformation circulating on social media can be classified into safety myths, false claims about vaccine ingredients, decontextualized adverse events, conspiracy narratives, anti-government discourse, anti-pharmaceutical industry discourse, false prevention or treatment alternatives, exploitation of scientific uncertainty, and misleading personal testimonies. Disinformation emerges when such content is deliberately produced or circulated and represents a more organized area of risk for public health communication. A longitudinal analysis of COVID-19 vaccine misinformation tracked on Twitter over one year showed that the intensity of misinformation increased simultaneously with events such as the emergence of new variants and policy changes (Pierri et al., 2023). A content analysis of Arabic Twitter content also found that a large proportion of misinformation consisted of conspiracy-themed and anti-government discourse (Al-Rawi et al., 2022). The way in which such content spreads is as important as its types. Emotional content, fear-based appeals, the amplifying effect of influential individuals, group identity, algorithmic recommendation systems, bots, coordinated campaigns, cross-platform sharing, and visual misinformation are the main mechanisms of diffusion. An analysis of bot activity in Spanish-language COVID-19 pro- and anti-vaccination networks showed that bots were particularly active in anti-vaccination networks (Ruiz-Nunez et al., 2022). The transfer of vaccine-related content from YouTube to Twitter and Facebook shows that misinformation does not remain confined to a single platform, but is reframed through cross-platform circulation (Ginossar et al., 2022). The public health implications of this multilayered diffusion pattern are observed in relation to trust, risk perception, vaccine confidence, vaccination intention, health behaviors, and adherence to public health measures. A retrospective study examining the impact of fake news on COVID-19 vaccination in Taiwan showed that intensive exposure was associated with measurable decreases in vaccination rates (Chen et al., 2022). The finding that low trust in social media was associated with lower COVID-19 vaccination rates and excess mortality indicates that misinformation is not merely a matter of communication quality, but a public health risk that can have potentially fatal consequences (Chen et al., 2023b). However, causality, inconsistencies in definitions of misinformation, platform restrictions on data access, language-based bias, and limited offline behavioral validation should be taken into account when interpreting these findings. A case study from Pennsylvania that tracked Twitter pandemic narratives using computational network science showed that localized misinformation patterns may differ from national-level generalizations (Alieva et al., 2023). This suggests that

assessments of misinformation effects should not rely on a single cross-sectional dataset and should be supported by social listening and infoveillance infrastructures.

Social Listening and Infoveillance

Social media data are used to monitor vaccine-related concerns, identify emerging rumors, track public sentiment, detect increases in misinformation, evaluate campaign responses, and inform message adaptation. Infoveillance refers to the systematic monitoring and analysis of these data to support public health decision-making (Teng et al., 2022). Among the methods used in this field, keyword monitoring, hashtag tracking, sentiment analysis, topic modeling, network analysis, bot detection, geospatial analysis, and time-series analysis stand out. The extraction of consumer insights using natural language processing techniques (Wang et al., 2023) and deep learning-based sentiment and stance analyses (Kucuk & Arici, 2023) are examples of this methodological diversity. Emotion analysis of COVID-19 vaccines using a fuzzy convolutional neural network also demonstrates that computational methods are becoming increasingly sophisticated (Qiu et al., 2024). Testing these methods in different linguistic and cultural contexts is important. In Canada, public attitudes toward COVID-19 vaccination were tracked on Twitter using machine learning (Jang et al., 2022). In the United Kingdom, emotional states and discussion topics during the pandemic were analyzed using geolocation data and text mining methods (Alhuzali et al., 2022). In Serbia, the reasons behind vaccine hesitancy were identified through sentiment-based topic modeling (Ljajic et al., 2022). French-language tweets were examined using a supervised learning approach (Sauvayre et al., 2022). The ultimate aim of this methodological richness is not only to produce academic observation, but also to generate actionable public health outputs. Infoveillance data are used in applications such as establishing early warning systems for misinformation, designing rapid response communication, segmenting target audiences, planning crisis communication, and developing personalized vaccine messages. The automated social media monitoring application presented at a digital health summit in Australia provides an example of how this approach can be operationalized at the institutional level (Habibabadi et al., 2023). Such monitoring systems are directly informed by institutions' efforts to make sense of online vaccine discourse through big data-based analyses; early signals enable the design of rapid response communication against misinformation surges (Teng et al., 2022). Nevertheless, infoveillance should be regarded not as a passive observational tool, but as an active public health component that provides input into message adaptation and intervention design. However, data access restrictions, API changes, lack of demographic information, samples with low representativeness, ethical issues arising from the monitoring of public discourse, and reproducibility problems limit this potential.

The fragility of social listening infrastructure also makes it more difficult to translate inveillance data directly into intervention design.

Social Media Based Vaccination Interventions

The findings produced by social listening and inveillance studies guide the design of social media-based interventions. Social media-based interventions can be classified as educational campaigns, misinformation correction and debunking, prebunking-based messaging, peer-led communication, health professional-led messaging, influencer-based campaigns, community engagement interventions, interactive question-and-answer applications, narrative and testimonial-based interventions, targeted advertising, and chatbot-supported communication. A study examining chatbot message preferences for COVID-19 vaccine communication among young adults showed that interactive artificial intelligence tools are a preferred communication format for younger populations (Weeks et al., 2022a). In a thematic analysis of COVID-19 messaging for American Indian and Alaska Native communities, the central role of cultural sensitivity in intervention design was emphasized (Weeks et al., 2022b). Culturally and linguistically congruent digital storytelling interventions developed to promote HPV vaccination also indicate that narrative-based approaches may be particularly functional in minority communities (Chen et al., 2023a; Kim et al., 2023). The “Brigada Digital de Salud” digital community health worker model, developed to inform Spanish-speaking communities against COVID-19 misinformation, showed that community-based digital interventions can increase reach and engagement (Andrade et al., 2023). The social media micro-influencer intervention developed for underserved communities in Tennessee represents a similar strategy (Hohmeier et al., 2022).

Outcome measures used to evaluate the effectiveness of these interventions vary across knowledge level, attitudes, beliefs, risk perception, vaccine confidence, vaccination intention, appointment scheduling, vaccination rate, and engagement metrics. A study examining the effects of social media-based social comparison information and similarity framing on vaccination behavior showed that message framing can generate meaningful effects on intention (Herzog et al., 2023). A large-scale field experiment conducted in the United States demonstrated that digital advertising-based interventions can reach many people simultaneously at low cost and that message content can be rapidly adapted (Athey et al., 2023). However, this positive picture should be interpreted cautiously in terms of the level of evidence. Some intervention studies rely on short follow-up periods, self-reported outcomes, and attitude or intention measures rather than behavior. The limited number of randomized controlled trials, insufficient evidence on actual vaccination rates, difficulty separating the effects of social media from broader communication exposure, and the limited generalizability of platform-specific findings are the main limitations (Elkin et

al., 2023; Rasmussen et al., 2024). Therefore, social media-based interventions should be evaluated not primarily by reach and engagement success, but by behavioral outcomes and their effect on reducing inequalities.

Social Media Platforms and Vaccination

Twitter/X is the most widely used platform for sentiment analysis, hashtag tracking, network analysis, political discourse analysis, and studies on misinformation diffusion. A retrospective study examining the rhetoric of public figures on Twitter regarding vaccination made visible, at the platform level, the relationship between the discourse of public figures and vaccine hesitancy (Honcharov et al., 2023). In Scandinavian countries, vaccine messages on Twitter were analyzed in local languages (Wahl et al., 2022). Facebook, by contrast, has a different user base and content dynamic. It is frequently used for studies of discussions in parenting groups, community-based vaccine discourse, public health campaigns, and group-based misinformation. In Malaysia, COVID-19 anti-vaccine sentiments were examined through narrative analysis of comments on Facebook posts (Wong et al., 2023). In addition, the impact of persuasive Facebook campaigns on national vaccination coverage was evaluated (Winters et al., 2023). These studies contribute to the development of a Facebook-specific evidence base. Observational examination of COVID-19 vaccine fact-checking posts on Facebook also demonstrates the potential of this platform for combating misinformation (Xue et al., 2022). In contrast, difficulty accessing private group data, ethical concerns, and limited platform transparency are among the main constraints of Facebook-based research.

Visual and video-based platforms further diversify the ways in which misinformation spreads. YouTube is used for video-based misinformation analysis, source credibility studies, comment analysis, and research on search and recommendation systems. Studies examining COVID-19 vaccine misinformation content on YouTube (Humprecht & Kessler, 2024), the impact of toxic comments on anti-vaccine YouTube videos (Miyazaki et al., 2024), and potential exposure to anti-vaccine videos from Facebook to YouTube (Gruzd et al., 2023) constitute the evidence base for this platform. The lack of transparency in YouTube's recommendation algorithms, and the possibility that videos may change, be removed, or be reclassified over time, are key limitations of research on this platform. Visually oriented platforms such as Instagram and TikTok have gained importance for vaccine messaging targeting young people, influencer communication, and short video analysis. Content assessment of COVID-19 vaccine communication on Instagram among young adults and its relationship with vaccination rates (Ford et al., 2024), cross-sectional examination of COVID-19 vaccine communication and misinformation on TikTok (van Kampen et al., 2022), and analysis of disinformation strategies in Instagram posts by European far-right parties (Sanchez-Castillo et al., 2023) indicate that the evidence base for

these newer platforms is still maturing. Difficulties in archiving dynamic visual content and the limited availability of standardized methods are the main methodological constraints specific to these platforms. Closed or semi-private messaging applications such as WhatsApp and Telegram provide more limited access for researchers. Due to restricted access, ethical and privacy concerns, and difficulties in sampling, the literature on these platforms remains limited compared with other platforms. Nevertheless, the examination of COVID-19 vaccine-related information on WeChat through topic modeling and content analysis shows that systematic research is also possible in closed or semi-closed digital environments (Wu et al., 2023).

Conclusion

The use of social media in public health indicates that vaccination, vaccine hesitancy, and digital infodemic should not be evaluated as independent topics, but as an interconnected whole in which each component mutually shapes the others. Social media provides public health institutions with a rapid, low-cost, broad-reaching, and interactive communication space. At the same time, social media creates a dynamic risk environment in which vaccine-related concerns, trust problems, misinformation, and disinformation become visible. Therefore, in vaccination communication, social media is not merely a technical channel through which messages are disseminated; it is also a digital public health space in which trust is built, risk perception is shaped, and vaccine decisions are socially negotiated.

The studies show that vaccine hesitancy in digital environments cannot be explained solely by a lack of information. Hesitancy is shaped together with institutional trust, access to scientific information, previous health experiences, political identity, conspiracy beliefs, platform algorithms, social norms, and cultural context. Therefore, combating digital infodemic should not be reduced to correcting misinformation alone. Misinformation correction should be integrated with social listening, infoveillance, target audience segmentation, selection of trusted messengers, and community-based communication strategies. Although social media-based interventions can produce positive effects on knowledge, attitudes, and intentions, these effects should be supported by digital communication, access to health services, and face-to-face counseling channels in order to translate into sustained vaccination behavior.

Important gaps remain in the existing literature. Most studies focus on COVID-19 vaccines. Routine childhood vaccines, HPV vaccination, influenza, vaccination during pregnancy, adult vaccines, and vaccines against emerging infections are addressed more limitedly. In addition, most studies are cross-sectional, platform-specific, or based on engagement metrics. Longitudinal designs, analyses linked to actual vaccination records, randomized interventions, and studies measuring behavioral outcomes remain relatively scarce. Low- and

middle-income countries, closed messaging applications, visual/video-based platforms, algorithmic recommendation systems, bot networks, and multilingual digital infodemic dynamics are also areas requiring further research.

From the perspective of public health practice, the main recommendation is that social media management should not be viewed as being limited to temporary campaigns during crisis periods. Continuous social listening and infoveillance capacity should be established within health institutions. In addition, early warning systems for misinformation and disinformation and platform-specific communication strategies should be developed. Messages should be designed not only in terms of scientific accuracy, but also in terms of comprehensibility, cultural appropriateness, linguistic accessibility, use of trusted sources, and the capacity to respond to the concerns of the target group. Health care workers, local opinion leaders, community representatives, and digital content creators should be included in the process as trusted actors in vaccine communication.

In conclusion, the use of social media in public health generates both a strategic opportunity for vaccination programs and a digital infodemic risk that must be managed. Reducing vaccine hesitancy and strengthening vaccine confidence are possible through the sharing of accurate information and the establishment of trust-based, participatory, platform-sensitive, and evidence-based communication ecosystems. Therefore, in future public health strategies, social media should be positioned beyond an auxiliary announcement channel as a strategic digital public health tool that supports the planning, monitoring, implementation, and evaluation of vaccination policies.

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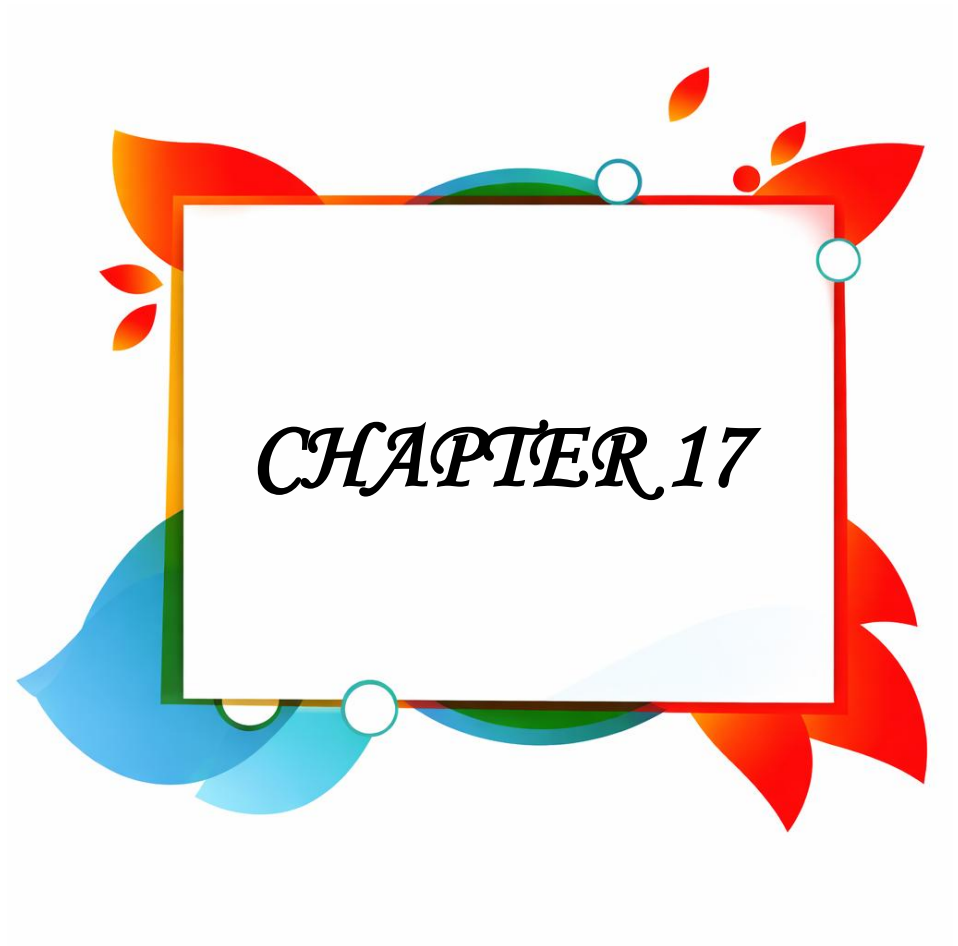
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Complications Following Pancreatic Surgery: Prevention and Management

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1. Introduction

Pancreatic surgery has evolved from a high-risk experimental discipline into a mature subspecialty of abdominal surgery. Technical refinements, high-volume center experience, improved anesthesia, structured perioperative care, and the widespread availability of interventional radiology have reduced mortality after pancreaticoduodenectomy and distal pancreatectomy. However, morbidity has not declined at the same pace, and major complications continue to shape postoperative recovery, hospital costs, readmission, and long-term oncological outcomes (Shrikhande et al., 2016; Pedrazzoli, 2017).

The pancreas is particularly unforgiving because its exocrine secretion contains proteolytic enzymes capable of digesting surrounding tissues when leakage occurs. In addition, the gland is anatomically close to the portal vein, superior mesenteric vein, hepatic artery, gastroduodenal artery stump, and splenic vessels. As a result, a small anastomotic leak or infected collection may progress to abscess, pseudoaneurysm, delayed hemorrhage, or multiorgan failure. This biological behavior explains why postoperative pancreatic fistula is considered the hallmark complication of pancreatic surgery (Bassi et al., 2017; Pulvirenti et al., 2018).

Contemporary management is no longer limited to the binary choice between observation and reoperation. A modern pancreatic surgery unit requires coordinated collaboration among surgeons, gastroenterologists, interventional radiologists, anesthesiologists, intensivists, infectious disease specialists, dietitians, and specialized nursing teams. This multidisciplinary structure allows early recognition of deterioration, appropriate interpretation of drain amylase and inflammatory markers, rapid CT assessment, timely drainage, and selective use of endovascular or endoscopic procedures (Addeo et al., 2020; Malleo & Vollmer, 2016).

The standardization of terminology has been one of the most important advances in the field. ISGPS definitions for postoperative pancreatic fistula,

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delayed gastric emptying, postpancreatectomy hemorrhage, and chyle leak have created a common language for clinical care and research. These definitions also help separate biochemical or clinically insignificant findings from complications that require a change in management (Wente et al., 2007a; Wente et al., 2007b; Bassi et al., 2017; Besselink et al., 2017).

2. Overview of Pancreatic Surgical Procedures

Pancreatic operations are performed for malignant, premalignant, inflammatory, traumatic, and selected benign diseases. The procedure type, gland texture, duct diameter, indication for surgery, vascular involvement, and reconstruction method determine the postoperative risk profile. A complication after pancreaticoduodenectomy may behave differently from the same named complication after distal pancreatectomy because the anatomy, reconstruction, and potential sources of contamination differ.

Pancreaticoduodenectomy remains the reference operation for tumors of the pancreatic head, distal bile duct, ampulla, and periampullary region. The classic or pylorus-preserving operation includes resection of the pancreatic head and duodenum followed by reconstruction using a pancreaticojejunostomy or pancreaticogastrostomy, hepaticojejunostomy, and gastrojejunostomy or duodenojejunostomy. The most relevant postoperative problems are pancreatic fistula, delayed gastric emptying, hemorrhage, bile leak, intra-abdominal infection, and nutritional impairment (Shrikhande et al., 2016; Kawai & Yamaue, 2010).

Distal pancreatectomy is usually performed for lesions in the pancreatic body or tail. It may include splenectomy or splenic preservation depending on oncological indication and technical feasibility. Although the operation avoids a pancreaticoenteric anastomosis, the transection surface may still leak pancreatic juice. Clinically relevant fistula therefore remains an important source of morbidity after distal pancreatectomy (Hackert & Büchler, 2012; van Dijk et al., 2017).

Total pancreatectomy eliminates the risk of pancreatic fistula but creates permanent endocrine and exocrine insufficiency. Brittle diabetes, malabsorption, weight loss, and quality-of-life issues become the dominant postoperative concerns. For this reason, total pancreatectomy should be individualized and reserved for appropriate indications such as multifocal disease, extensive ductal involvement, or situations in which safe reconstruction is not feasible.

Minimally invasive pancreatic surgery, including laparoscopic and robotic techniques, has expanded in specialized centers. Potential advantages include reduced blood loss, smaller incisions, earlier mobilization, and shorter hospital stay in selected patients. Nevertheless, pancreatic surgery remains technically demanding, and safe implementation depends on training, case volume,

appropriate patient selection, and institutional rescue capacity (Marchegiani et al., 2018; Asbun et al., 2020; Kamarajah et al., 2020).

3. Classification and General Principles of Complication Management

Postoperative complications should be described using standardized, clinically meaningful systems. The Clavien-Dindo classification remains useful for grading treatment intensity, whereas ISGPS definitions provide pancreas-specific interpretation. In practice, a complication becomes clinically relevant when it changes the postoperative course by requiring antibiotics, prolonged drainage, nutritional support, radiological or endoscopic intervention, intensive care, reoperation, or readmission (Tol et al., 2020).

The first principle is early recognition. Subtle findings such as persistent tachycardia, rising C-reactive protein, unexpected ileus, low-grade fever, change in drain color, delayed mobilization, or unexplained abdominal discomfort may precede overt sepsis or hemorrhage. A patient who is not following the expected recovery curve after pancreatic surgery deserves structured reassessment rather than passive observation.

The second principle is source control. In pancreatic surgery, source control often means maintaining or establishing adequate drainage of pancreatic juice, bile, blood, or infected collections. CT-guided drainage, endoscopic ultrasound-guided drainage, and angiographic treatment can convert a potentially fatal complication into a controlled postoperative problem (Addeo et al., 2020; Malleo & Vollmer, 2016).

The third principle is nutritional and physiological support. Catabolism, systemic inflammation, delayed oral intake, and pancreatic exocrine insufficiency frequently overlap. Nutritional treatment should be proactive rather than delayed until severe malnutrition is obvious. Enteral nutrition is generally preferred when the gastrointestinal tract is functional, while parenteral nutrition remains valuable when enteral feeding is not possible or insufficient (Gianotti et al., 2018).

4. Postoperative Pancreatic Fistula

Postoperative pancreatic fistula is the central complication around which many other postoperative events revolve. The 2016 ISGPS update redefined clinically relevant POPF and distinguished it from biochemical leak. A clinically relevant fistula requires a drain amylase value greater than three times the upper normal serum level with a condition that affects patient management. This distinction is clinically important because not every amylase-rich drain output represents a complication that requires aggressive treatment (Bassi et al., 2017).

Risk factors for POPF include soft pancreatic texture, small main pancreatic duct diameter, high-risk pathology such as ampullary or neuroendocrine tumors, obesity, malnutrition, increased intraoperative blood loss, and technically

difficult reconstruction. Fistula risk scores were developed to make these risks more objective and to support individualized drain and postoperative strategies (Ansari et al., 2017; Mungroop et al., 2019).

The clinical spectrum is broad. A biochemical leak may have no clinical consequence, whereas grade B or C fistula can lead to abscess, sepsis, delayed gastric emptying, hemorrhage, organ failure, or death. Persistent fever, leukocytosis, abdominal pain, turbid drain output, delayed recovery, and rising inflammatory markers should raise suspicion. CT is frequently required to identify undrained collections and guide intervention.

Prevention begins before the anastomosis. Preoperative nutritional assessment, correction of severe hypoalbuminemia when possible, careful operative planning, gentle tissue handling, accurate duct identification, and meticulous reconstruction are essential. Numerous anastomotic techniques have been proposed, including duct-to-mucosa pancreaticojejunostomy, invagination techniques, pancreaticogastrostomy, internal stenting, and external duct stenting. No single method eliminates fistula risk in all patients; therefore, the optimal technique should be adapted to pancreatic texture, duct size, surgeon experience, and institutional outcomes (Kawai & Yamaue, 2010; Klaiber et al., 2018).

Pharmacological prevention has been investigated for many years. Somatostatin analogues reduce exocrine secretion theoretically, but clinical results vary according to agent, population, and institutional practice. Pasireotide demonstrated benefit in one randomized trial, whereas broader evidence remains mixed and routine use should be interpreted within local risk profiles and cost considerations (Allen et al., 2014; Klaiber et al., 2018).

Management follows a step-up strategy. Stable patients with controlled external drainage can usually be managed with observation, maintenance of drainage, antibiotics when infection is suspected, glycemic control, and nutritional support. Drain removal should not be based only on calendar time; drain amylase, output volume, appearance, clinical condition, and imaging findings must be considered together.

Percutaneous drainage is appropriate when imaging demonstrates a symptomatic or infected collection that is not adequately drained by surgical drains. Catheter position may require adjustment, upsizing, or repeated drainage. Endoscopic drainage may be considered for selected collections accessible from the stomach or duodenum. Reoperation is reserved for uncontrolled sepsis, complete anastomotic disruption, necrosis, or hemorrhage not manageable by less invasive methods (Addeo et al., 2020).

5. Delayed Gastric Emptying

Delayed gastric emptying is a frequent cause of prolonged recovery after pancreaticoduodenectomy. The ISGPS definition classifies DGE according to

nasogastric tube requirement, inability to tolerate solid oral intake, vomiting, and the need for prokinetic medication. The definition is useful because it separates transient functional delay from clinically significant impairment that delays discharge (Wente et al., 2007a; Connor, 2019).

DGE is often multifactorial. Proposed mechanisms include pyloric edema or dysfunction, vagal denervation, inflammation around the anastomoses, ischemia, postoperative pancreatitis, intra-abdominal abscess, pancreatic fistula, and the effect of opioids. Importantly, DGE may be a primary functional problem or a secondary manifestation of another complication. Therefore, persistent DGE should trigger a search for POPF, abscess, bile leak, or intra-abdominal collection rather than being treated as an isolated problem.

Clinical features include nausea, vomiting, abdominal distention, inability to tolerate oral intake, and prolonged nasogastric decompression. Management is usually conservative. Correction of electrolyte imbalance, reduction of opioid use, prokinetic therapy, mobilization, and nutritional support are central. Enteral feeding through a jejunal route may be useful when prolonged intolerance is expected. Parenteral nutrition should be reserved for situations in which enteral feeding is not feasible or insufficient.

Prevention depends on minimizing intra-abdominal complications, ensuring tension-free reconstruction, reducing postoperative opioids through multimodal analgesia, and applying ERAS principles. The debate regarding pylorus preservation versus antrectomy continues, but the current practical approach is to focus on safe reconstruction and early detection of associated complications rather than assuming that one anatomical technique alone prevents DGE (Connor, 2019; Melloul et al., 2020).

6. Postpancreatectomy Hemorrhage

Postpancreatectomy hemorrhage is among the most dangerous complications after pancreatic surgery. It may occur early, usually within the first 24 hours, or late, often after postoperative day 5. The ISGPS definition classifies PPH according to timing, location, severity, and clinical impact. This classification is valuable because delayed hemorrhage has a different pathophysiology and prognosis from immediate technical bleeding (Wente et al., 2007b).

Early hemorrhage is often related to inadequate hemostasis, coagulopathy, or technical failure. In contrast, delayed hemorrhage is frequently associated with POPF, abscess, enzymatic erosion of arterial walls, or pseudoaneurysm formation. Sentinel bleeding from drains, melena, hematemesis, or a small unexplained hemoglobin drop may precede massive hemorrhage. These warning signs should never be ignored in a patient after pancreatic resection.

Initial management requires hemodynamic assessment, resuscitation, blood product preparation, correction of coagulopathy, and urgent localization of

bleeding. In stable or temporarily stabilized patients, CT angiography is usually the key diagnostic test. It may identify active extravasation, pseudoaneurysm, hematoma, or inflammatory changes surrounding the arterial stump.

Endovascular treatment has become the preferred strategy for many cases of delayed PPH. Selective arterial embolization, coil placement, glue embolization, and covered stent placement may control bleeding while avoiding the morbidity of emergency relaparotomy. The decision between embolization and stenting depends on vessel anatomy, collateral circulation, hepatic arterial flow, and institutional expertise (Malleo & Vollmer, 2016).

Surgery remains essential when the patient is unstable, endovascular treatment is unavailable or unsuccessful, or bleeding is associated with uncontrolled sepsis and abdominal catastrophe. Operative options include packing, hematoma evacuation, vessel ligation, revision of anastomoses, completion pancreatectomy, and damage-control surgery. Because operative mortality can be high in this setting, early diagnosis and rapid endovascular access are critical (Malleo & Vollmer, 2016).

7. Intra-Abdominal Collections and Abscesses

Intra-abdominal collections are common after pancreatic surgery and may arise from pancreatic fistula, bile leak, lymphatic leakage, hemorrhage, or infected postoperative fluid. The clinical significance depends on location, size, infection, symptoms, and whether adequate drainage exists. Small sterile collections may resolve spontaneously, whereas infected collections may lead to sepsis or hemorrhage.

CT is the primary imaging modality for evaluation. It helps define collection size, gas content, relation to anastomoses and vessels, and feasibility of percutaneous drainage. Repeated imaging should be driven by clinical course rather than routine scheduling. Rising inflammatory markers, fever, persistent ileus, abdominal pain, and failure to progress are common indications for reassessment (Addeo et al., 2020).

Image-guided percutaneous drainage is usually the first-line intervention for symptomatic or infected collections. Drainage should be combined with microbiological culture and targeted antibiotics. Catheter management is an active process; flushing, repositioning, upsizing, and additional drains may be needed. Multiloculated collections or collections adjacent to major vessels require careful planning.

Endoscopic drainage may be useful when a collection is adjacent to the stomach or duodenum and accessible by EUS. Surgical drainage is now reserved for failed minimally invasive treatment, generalized peritonitis, associated bowel injury, or uncontrolled sepsis. This step-up strategy has reduced the need for

emergency reoperation and is now integral to modern pancreatic complication management (Addeo et al., 2020; Malleo & Vollmer, 2016).

8. Biliary Leakage and Chyle Leak

Biliary leakage after pancreaticoduodenectomy usually arises from the hepaticojejunostomy. Although less frequent than pancreatic fistula, it may contribute to abscess, peritonitis, sepsis, and prolonged hospitalization. The diagnosis is suspected when bilious fluid is observed in drains or when biochemical analysis confirms elevated bilirubin concentration in drain fluid.

Minor biliary leaks often resolve with adequate external drainage and supportive care. Persistent leakage may require percutaneous transhepatic biliary drainage, endoscopic intervention when anatomically feasible, or rarely surgical revision. As with pancreatic fistula, the goal is controlled drainage, prevention of undrained infected collections, and avoidance of unnecessary reoperation.

Chyle leak is another recognized complication after pancreatic surgery, particularly when extensive lymphadenectomy or vascular dissection has been performed. ISGPS proposed a definition and grading system that helps distinguish clinically relevant leaks from low-output lymphatic drainage. Management includes dietary fat restriction, medium-chain triglycerides, enteral or parenteral nutritional modification, somatostatin analogues in selected cases, and rarely lymphatic intervention or surgery (Besselink et al., 2017).

9. Surgical Site Infection and Infectious Complications

Surgical site infections remain common after pancreatic procedures because of long operative time, biliary colonization, preoperative biliary drainage, malnutrition, diabetes, and anastomotic leakage. Infections may be superficial, deep incisional, or organ-space infections. Organ-space infection is particularly important because it is often linked to POPF, bile leak, or undrained collections (de Pastena et al., 2019).

Prevention requires a bundle approach rather than a single intervention. Appropriate antibiotic prophylaxis, attention to bile culture results when available, normothermia, glucose control, careful tissue handling, wound protection, and avoidance of unnecessary transfusion all contribute. In patients with biliary stents, bacterial colonization may alter prophylactic strategies and postoperative antibiotic decisions.

Treatment depends on depth and source. Superficial infections generally require opening of the wound, local care, and selective antibiotics. Deep or organ-space infections require imaging, source control, and targeted antimicrobial therapy. Prolonged broad-spectrum antibiotics without drainage of an infected collection is rarely sufficient and may increase resistant organisms or fungal complications (de Pastena et al., 2019; Addeo et al., 2020).

10. Nutritional and Metabolic Complications

Nutritional impairment is common before and after pancreatic surgery. Malignancy-related cachexia, biliary obstruction, gastric outlet symptoms, systemic inflammation, decreased oral intake, and pancreatic exocrine insufficiency may coexist. Postoperatively, delayed gastric emptying, fistula, infection, and prolonged catabolism further increase nutritional risk.

Early nutritional screening should be part of routine perioperative assessment. Serum albumin alone is not a complete nutritional marker, but hypoalbuminemia may reflect systemic inflammation and increased complication risk. Dietitian involvement, prehabilitation, and individualized nutritional planning are especially important in frail, sarcopenic, jaundiced, or elderly patients (Gianotti et al., 2018).

Enteral nutrition is generally preferred when tolerated because it maintains intestinal barrier function and is associated with fewer infectious complications than prolonged exclusive parenteral nutrition. Oral feeding can often be resumed early within ERAS pathways when there is no contraindication. When oral intake is insufficient, enteral tube feeding or parenteral supplementation should be considered according to clinical condition (Gianotti et al., 2018; Melloul et al., 2020).

Exocrine pancreatic insufficiency may present with steatorrhea, bloating, weight loss, fat-soluble vitamin deficiency, and poor recovery. Pancreatic enzyme replacement therapy should be used when symptoms or objective evidence suggest malabsorption. Endocrine insufficiency requires careful glucose monitoring, especially after distal or total pancreatectomy. Total pancreatectomy creates permanent insulin-dependent diabetes and complete exocrine insufficiency, requiring structured long-term follow-up.

11. Role of Interventional Radiology and Endoscopy

Interventional radiology has become indispensable in pancreatic surgery. Many complications previously treated by emergency reoperation can now be managed with percutaneous drainage, angiographic embolization, covered stent placement, or percutaneous biliary intervention. This transformation has changed the risk-benefit calculation in postoperative decision-making (Malleo & Vollmer, 2016).

The success of interventional radiology depends on early consultation, high-quality imaging, and clear surgical communication. The surgeon must define the clinical problem: suspected infected collection, uncontrolled fistula, pseudoaneurysm, biliary obstruction, or hemorrhage. The radiologist contributes by determining the safest access route, catheter strategy, embolization target, and need for follow-up procedures.

Endoscopic interventions are increasingly used for selected postoperative complications. EUS-guided drainage, pancreatic duct stenting, endoscopic hemostasis, and management of luminal obstruction may reduce the need for surgery in carefully selected cases. However, altered anatomy after pancreaticoduodenectomy can limit access and requires advanced expertise.

The key concept is not that minimally invasive treatment replaces surgery, but that it expands the therapeutic pathway. Successful management depends on choosing the right intervention at the right time for the right patient. Reoperation should remain available as a rescue strategy, but it should not be the default response to every postoperative deterioration (Malleo & Vollmer, 2016).

12. Enhanced Recovery After Surgery Protocols

ERAS protocols aim to reduce surgical stress, accelerate functional recovery, and standardize perioperative care. In pancreatic surgery, ERAS components include preoperative counseling, optimization of nutrition, avoidance of prolonged fasting, multimodal opioid-sparing analgesia, early mobilization, early oral or enteral intake, glycemic control, rational fluid therapy, and standardized drain and tube management (Melloul et al., 2020).

Implementation requires institutional commitment. A written pathway alone is insufficient if nursing routines, anesthesia practice, surgical decision-making, dietitian support, and discharge planning are not aligned. Daily goal-directed postoperative care helps detect deviation from expected recovery and may reveal complications earlier.

ERAS should be applied flexibly rather than mechanically. A patient with POPF, sepsis, hemorrhage, or severe DGE should not be forced through a pathway designed for uncomplicated recovery. The value of ERAS is to create a structured baseline while allowing rapid escalation when complications occur (Melloul et al., 2020).

13. Minimally Invasive and Robotic Pancreatic Surgery

Minimally invasive pancreatic surgery has expanded with advances in laparoscopic and robotic systems. Distal pancreatectomy is more widely adopted than minimally invasive pancreaticoduodenectomy because it avoids complex pancreatocenteric reconstruction. Robotic platforms may facilitate suturing, fine dissection, and ergonomics, but they also introduce cost, learning curve, and team-training considerations (Kamarajah et al., 2020; Asbun et al., 2020).

The complication profile after minimally invasive surgery is not fundamentally different from open surgery. POPF, hemorrhage, DGE, and abscess remain relevant. The potential benefits are mainly related to reduced wound morbidity, lower blood loss, and faster functional recovery in selected

patients. However, oncological principles and safety must not be compromised for the sake of a smaller incision (Marchegiani et al., 2018; Asbun et al., 2020).

Patient selection is critical. Tumor location, vascular involvement, pancreatitis, obesity, previous surgery, and institutional experience must be considered. Safe adoption should follow structured training and should ideally occur in centers with established open pancreatic surgery expertise and rescue capacity.

14. Artificial Intelligence and Future Perspectives

Artificial intelligence and machine learning are increasingly investigated in pancreatic surgery. Potential applications include preoperative risk prediction, automated body composition assessment, radiomic evaluation of pancreatic texture, prediction of POPF, detection of early deterioration, and decision support for postoperative imaging or drainage (Ferrari et al., 2023).

The promise of artificial intelligence is not to replace clinical judgment but to enhance individualized risk stratification. For example, a model integrating pancreatic duct diameter, texture surrogates on imaging, body composition, laboratory values, operative variables, and early postoperative drain amylase may help identify patients suitable for early drain removal versus closer surveillance.

Several limitations remain. Models may perform poorly outside the population in which they were trained. Data quality, missing variables, interpretability, external validation, and integration into clinical workflow are major challenges. Future research should focus on transparent, clinically usable tools that improve decisions rather than simply generating statistical accuracy.

Centralization, multidisciplinary care, better perioperative optimization, and precision risk prediction are likely to define the next phase of pancreatic surgery. Technological innovation will be most valuable when combined with surgical expertise, standardized definitions, and high-quality postoperative rescue systems (Pedrazzoli, 2017; Ferrari et al., 2023).

15. Practical Recommendations for Clinical Practice

Major pancreatic resections should preferably be performed in centers with experienced teams, standardized pathways, and immediate access to intensive care, endoscopy, and interventional radiology. High-volume experience is associated with better recognition and management of complications, not merely lower operative mortality (Pedrazzoli, 2017).

Drain strategy should be individualized. Early removal may be safe in low-risk patients with favorable drain amylase and uncomplicated recovery, whereas high-risk patients require closer monitoring. A drain is helpful only when it controls a problem; an ineffective drain can create false reassurance. Drain

output, amylase level, appearance, culture results, and imaging findings should be interpreted together.

Every postoperative pancreatic patient should be assessed daily for deviation from the expected recovery curve. Persistent tachycardia, fever, delayed feeding, abdominal pain, altered drain fluid, rising inflammatory markers, or unexplained anemia should prompt structured evaluation. Early CT, drain revision, percutaneous drainage, or angiography can prevent progression to severe morbidity.

Reoperation should be neither delayed in a collapsing patient nor overused in a stable patient who can be rescued by minimally invasive means. The best outcomes usually arise from balanced judgment: aggressive diagnosis, early source control, appropriate escalation, and multidisciplinary communication.

16. Conclusion

Pancreatic surgery remains one of the most challenging fields in gastrointestinal surgery. Despite substantial improvements in mortality, postoperative complications remain common and clinically important. POPF, DGE, PPH, intra-abdominal collections, biliary leakage, infection, chyle leak, and nutritional-metabolic problems require early recognition and individualized management.

The modern approach is multidisciplinary and step-up based. Standardized ISGPS definitions, careful clinical surveillance, CT-guided drainage, endovascular hemorrhage control, advanced endoscopy, ERAS protocols, and nutritional support have reshaped postoperative care. Surgical reintervention remains essential in selected cases, but many complications can now be controlled without immediate relaparotomy.

Future improvements will depend on centralization of complex pancreatic surgery, better risk prediction, artificial intelligence-supported decision tools, and continued refinement of perioperative care. Ultimately, successful complication management depends on timely diagnosis, technical competence, institutional preparedness, and patient-centered clinical judgment.

Tables

Table 1. Common complications following pancreatic surgery

Complication	Typical onset	Clinical findings	First-line management
Postoperative pancreatic fistula	Postoperative day 3-7	Elevated drain amylase, fever, sepsis	Drainage, antibiotics, nutritional support
Delayed gastric emptying	Postoperative day 5-10	Vomiting, prolonged nasogastric tube requirement	Conservative treatment, prokinetics, nutrition
Postpancreatectomy hemorrhage	Early or late postoperative period	Sentinel bleeding, anemia, shock	Resuscitation, CT angiography, embolization
Intra-abdominal abscess	Variable	Fever, leukocytosis, abdominal pain	Antibiotics and image-guided drainage
Biliary leakage	Early postoperative period	Bilious drain output, collection	Drainage, biliary decompression when needed
Chyle leak	After enteral feeding begins	Milky drain output, nutritional loss	Dietary modification and drainage

Table 2. Major risk factors for postoperative pancreatic fistula

Patient-related factors	Disease-related factors	Operative factors
Obesity, malnutrition, sarcopenia	Soft gland, nonfibrotic pancreas	Small duct, high blood loss
Diabetes or impaired immunity	Ampullary or neuroendocrine tumors	Long operative time
Hypoalbuminemia	Absence of chronic pancreatitis	Difficult reconstruction

Table 3. Step-up management strategy for major complications

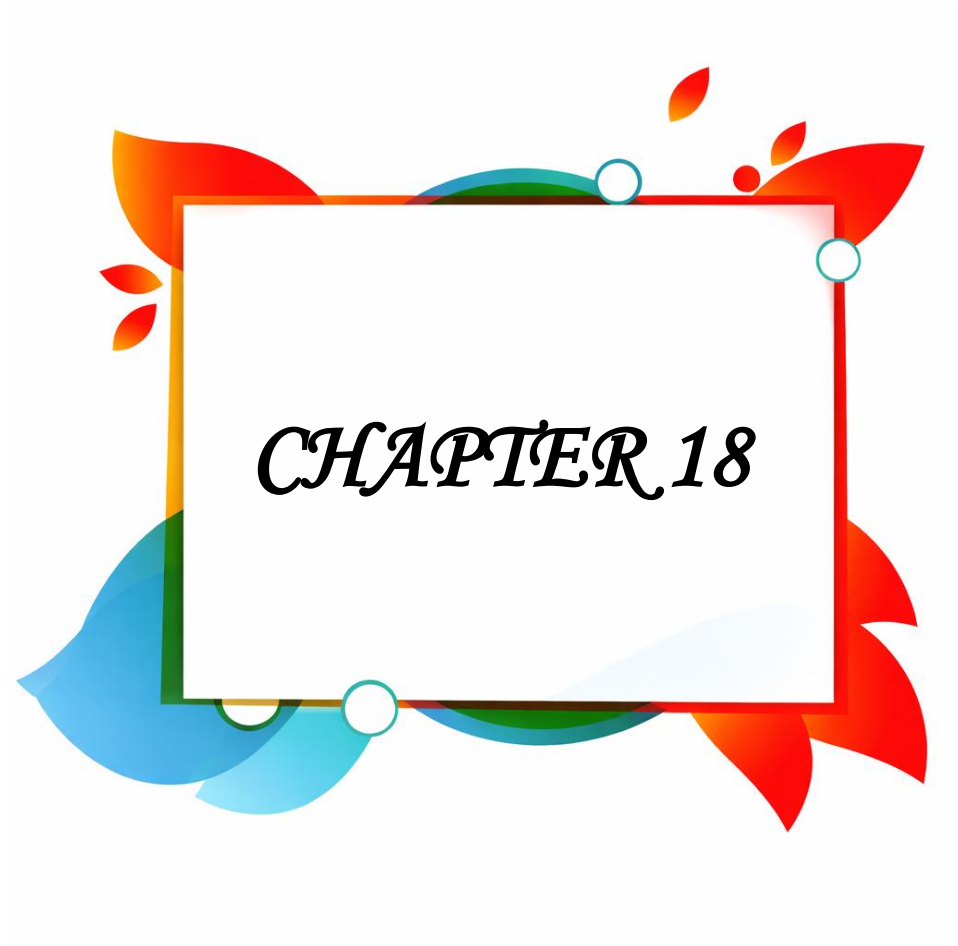
Complication	Conservative treatment	Interventional treatment	Surgical treatment
POPF	Drain maintenance, antibiotics, nutrition	Percutaneous or endoscopic drainage	Reoperation for uncontrolled sepsis or disruption
PPH	Resuscitation and correction of coagulopathy	Angiographic embolization or covered stent	Emergency exploration if unstable or failed embolization
Abscess	Antibiotics and monitoring	Image-guided drainage	Surgical drainage if failed minimally invasive control
Bile leak	Drainage and observation	Percutaneous biliary drainage	Revision rarely required

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Prosthetic Approaches in Pediatric Dentistry

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1. FIXED PROSTHESES

1.1. Direct Resin Crowns and Composite Strip Crowns

1.1.A. DIRECT RESIN CROWNS

The technique defined by Webber and colleagues (1979) relies on using a celluloid crown form as a mold during the restoration of the tooth with a resin-based composite material. The researchers reported that when the celluloid form is properly filled, the restoration naturally takes on the original contours of the tooth, often eliminating the need for extensive finishing and polishing procedures afterward (Webber, Epstein, Wong, & Mueller, 1979).

As shown in Figure 1, the method, also referred to as the "jacket crown technique," is based on the use of vacuum-formed celluloid molds. In the literature, this technique is now commonly referred to as the "strip crown" method.

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Figure 1.1.A (A) Extensive decay in the anterior maxillary incisors of a 4-year-old patient. (B) Celluloid crown forms tailored to fit the prepared teeth, trimmed to the cervical margin. (C) Placement of the forms filled with composite resin, removal of the excess material, and light polymerization. (D) Final polishing procedure. (Source: Dean, J.A., McDonald and Avery's Dentistry for the Child and Adolescent, 11th ed., 2021.)

A retrospective evaluation has shown a high clinical success rate for strip crowns in the restoration of primary incisors with large cavities or multiple-surface loss. Retention was maintained in more than 80% of 145 restorations, supporting the long-term durability of the method (Kupietzky, Waggoner, & Galea, 2005).

The use of celluloid crowns is not limited to the anterior region; they can also serve as a temporary solution in the rehabilitation of primary molars. Particularly in cases where ankylosed primary molars are in infraocclusion, reconstructing them with resin to preserve occlusion and arch integrity stands out as an important indication.

However, whether such restorations are always medically necessary remains open to debate. Lengthy and technically demanding procedures in early childhood can test a child's cooperation. Moreover, it should be remembered that these teeth will exfoliate physiologically within a short time. As noted by Sharaf (personal communication, 2019), these interventions can sometimes turn into unnecessary procedures driven primarily by aesthetic concerns.

Ultimately, it is the dentist's responsibility to evaluate the advantages and limitations of the method transparently with the parents and to jointly choose the approach most beneficial for the child.

1.1.B. COMPOSITE STRIP CROWNS

Early childhood caries is a rapidly progressive pathological process that particularly affects primary incisors in the maxillary anterior region. The resulting

aesthetic loss is not confined solely to the dental dimension; it creates significant psychosocial barriers affecting children's early confidence development, social acceptance processes, peer relationships, and even learning motivation. With the increasing awareness among families today, there is a growing demand for solutions that are both biomechanically robust and visually satisfactory for the restoration of anterior teeth affected by severe ECC.

However, achieving predictable and lasting aesthetic outcomes in primary incisors is a challenging process for clinicians. Behavioral difficulties frequently encountered in pediatric patients and the inability to achieve adequate moisture control (isolation) in the oral cavity are fundamental factors that negatively affect bonding quality and consequently the longevity of the restoration. In this context, although composite resin strip crowns shaped directly in the oral cavity initially promise superior aesthetic results, they possess clinical disadvantages including the material's moisture sensitivity, the technically demanding application phase, and difficulty maintaining ideal color harmony with adjacent teeth (Grewal, Jha, & Kaur, 2021).



Figure 1.1.B. (A–C) Follow-up of GC Equia Forte strip crown restorations applied to teeth 52 and 62; (D–F) Follow-up of GC G-aenial strip crown restorations applied to teeth 52 and 62 (Malik, Acharya, & Nayak, 2025).

Maxillary incisors represent the tooth group most susceptible to pathological processes due to the sequence of tooth eruption, muscle activity occurring during sucking movements in infancy, and their inability to adequately benefit from the natural protective function of saliva. Patients presenting with early childhood caries findings frequently exhibit Class III, IV, or extensive multi-surface lesions in this region (Muhamad, Azzaldeen, & Mai, 2021).

Composite resin strip crowns have long been employed as the primary method of aesthetic reconstruction in pediatric patients with low levels of cooperation. However, the success of this technique is directly dependent on advanced technical skill and the adequacy of the sound tooth structure remaining after removal of carious tissue. The majority of clinical failures stem from inappropriate patient selection, inadequate hemorrhage and moisture isolation, or errors during the preparation process (Lee, Bouwens, Savage, et al., 2006).

Furthermore, the lack of bioactive protective mechanisms such as fluoride release in conventional composite resins constitutes a notable disadvantage in high caries-risk cases such as ECC. Fluoride release and the capacity for fluoride recharging offer a significant advantage in preventing demineralization processes. In this context, glass ionomer cements (GIC) are considered a biologically more protective alternative owing to their chemical bonding capacity and fluoride release dynamics (Waggoner, 2015).

From an aesthetic standpoint, although composite systems initially provide satisfactory results, there is a risk of decreased surface luster and color degradation over time. While this change remains relatively limited in composites containing high-quality filler particles, "gray discoloration" may be observed in restorations exposed to moisture and particularly blood contamination, especially in areas near the gingival margin (Elbahary, Bercovich, Flaisher-Solemn, et al., 2020).

Additionally, the translucent character of composites may not always achieve complete harmony with the typical matte-white appearance of primary teeth. To address this optical discrepancy, supplementary use of resin-modified glass ionomer cement (RMGIC) or opacifying resins may sometimes be necessary (Lee, Bouwens, Savage, et al., 2006).

1.2. Bonded Composite Veneer and Bonded Laminate Veneer Restorations

1.2.A. BONDED COMPOSITE VENEER RESTORATIONS

Bonded composite veneer restorations represent one of the most effective examples of a minimally invasive approach in anterior teeth. This technique is based on mechanically and chemically bonding the resin-based material to an acid-etched enamel surface (Araujo & Perdigão, 2021).

Clinically, it is widely used for the treatment of fractured teeth, diastemas, hypoplastic defects, or discolorations. Preserving tooth form in young patients provides significant biological and aesthetic advantages (Demarco et al., 2015).

Its most notable advantage is enamel preservation. Treatment can often be completed in a single appointment, at low cost, and can be repaired directly in the mouth if needed (Peumans et al., 2000). However, potential drawbacks include reduced surface gloss and a tendency toward discoloration over time. With careful technique and regular maintenance, these disadvantages can be minimized.

As with any restorative method, bonded composite veneers have limitations. Compared with ceramic restorations, they may show reduced surface gloss, marginal staining, or wear over the long term. Nonetheless, with proper case selection, careful adhesive procedures, and regular checkups, these issues can be significantly mitigated (Demarco et al., 2015).

Overall, when performed with precision and under the correct indication, bonded composite veneers offer a conservative and aesthetically satisfying treatment option in modern restorative dentistry.

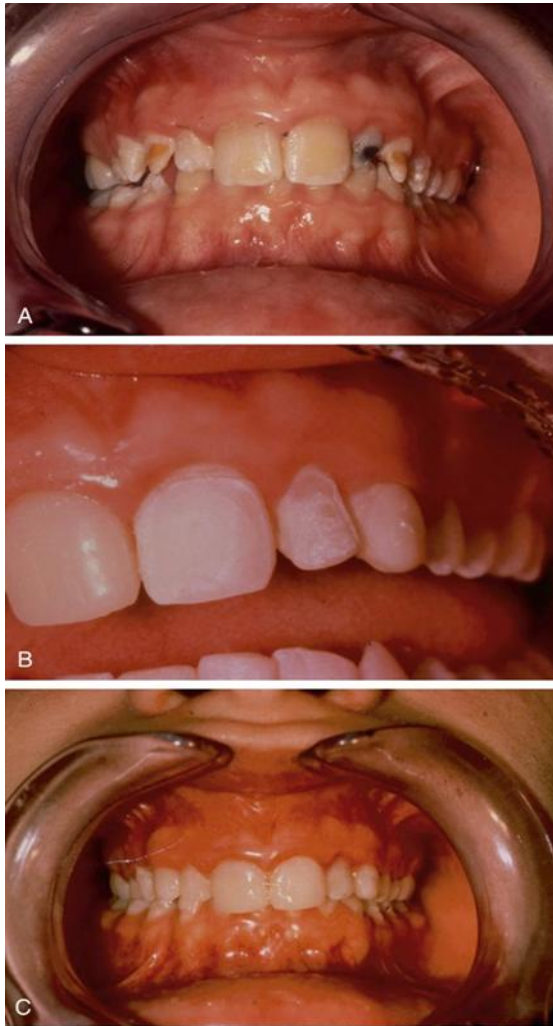


Figure 1.2.A. (A) A 15-year-old patient dissatisfied with the appearance of anterior teeth. (B) Preparations of teeth 21, 22, and 23, followed by adhesive application of a highly filled composite. (C) Post-treatment appearance of the final restorations. (Dean, J.A., 2021)

1.2.B. BONDED PORCELAIN LAMINATE VENEER RESTORATIONS

Porcelain laminate veneers are conservative restorations widely used to correct aesthetic and functional problems in anterior teeth. The basic principle of this technique is to preserve as much enamel as possible while achieving a strong adhesive bond (Gurel, 2003; Peumans et al., 2000).

This method offers high predictability in cases such as masking severe discolorations, correcting shape anomalies, and closing diastemas (Goodacre et al., 2008). Typically, enamel reduction is limited to 0.3–0.7 mm, with the finish

line designed as a light chamfer (Gurel, 2003).

Compared to composite alternatives, porcelain laminate veneers provide superior color stability, biocompatibility, and longevity. When proper case selection and ideal occlusal conditions are ensured, successful outcomes lasting over 10 years have been reported (Layton & Walton, 2007).

In conclusion, laminate veneer restorations are considered a reliable treatment option in modern aesthetic dentistry, providing high aesthetics and long-term success through a minimally invasive approach.

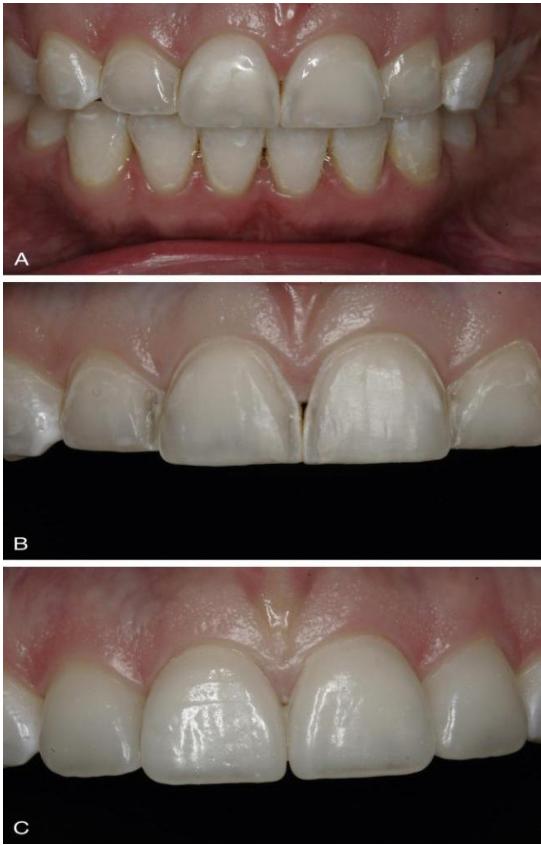


Figure 1.2.B. (A) Hypoplastic defects in anterior teeth after orthodontic treatment. (B) Appearance after intra-enamel preparation. (C) Bonded laminate veneers in place. (Dean, J.A., 2021)

1.3. Fiber-reinforced adhesive bridges

Because implant placement should be postponed until growth and development are complete, it is crucial to provide a temporary yet aesthetically acceptable solution for missing teeth in young individuals. Fiber-reinforced composite bridges (FRCBs) offer a conservative alternative that preserves tooth structure.

Originally used for periodontal splinting and stabilization of avulsed teeth (Iglesia-Puig & Arellano-Cabornero, 2003), fiber systems are now also used as temporary restorations for replacing missing anterior and posterior teeth (Wolff et al., 2011).

Successful outcomes depend on periodontal health of the abutment teeth, the absence of parafunctional habits, proper occlusal load distribution, and correct selection of fiber material (Isola et al., 2019; Perea-Lowery & Vallittu, 2018).

Fiber-reinforced bridges can be applied using two techniques:

Direct technique: Completed in a single appointment, eliminating the need for laboratory work and lowering cost. However, it requires clinical experience in handling and isolation (Singh et al., 2014).

Indirect technique: Involves a laboratory stage, is more costly, and requires additional appointments but generally provides better aesthetic harmony (Benito et al., 2012).

These systems are especially appealing for young patients as they contain no metal, are easily repairable, cost-effective, and maintain a natural tooth feel (Bagis et al., 2010).



Figure 1.1. (A) Posterior edentulous region. (B) Inlay-type preparation on proximal surfaces of adjacent teeth. (C) Schematic representation of the fiber-reinforced structure of the bridge. (D) Bonding of the fiber to teeth with adhesive system. (E) Formation of pontic and inlay surfaces using a silicone index. (F) Completed bridge after occlusal adjustment and polishing. (Dent. J., 2020)

1.4. Stainless Steel Crowns

Stainless steel crowns (SSCs) are among the most widely used restorative options in pediatric dentistry for repairing and protecting the remaining structure of teeth with extensive substance loss or those weakened by advanced caries. First described by Engel in 1947 and later popularized by Humphrey in 1950, SSCs have held a central place in pediatric practice for more than half a century. They have consistently demonstrated superior durability and longevity compared with other materials such as amalgam and composite. When a temporary full-coverage restoration is required, no alternative matches SSCs in terms of low cost, strength, and reliability (Seale, 2002; Engel, 1947; Humphrey, 1950).

Indications for Stainless Steel Crown Use (Seale, 2002; American Academy of Pediatric Dentistry, 2020; McDonald, Avery, & Dean, 2016)

- Teeth that have undergone pulp therapy
- Extensive carious lesions involving multiple surfaces
- Children at high caries risk
- Primary teeth with developmental disorders such as amelogenesis imperfecta, dentinogenesis imperfecta, or enamel hypoplasia
- Severely worn teeth
- Cases in which failure of the restoration is highly likely
- Fractured teeth
- Children who have difficulty cooperating during long treatment sessions

Contraindications for Stainless Steel Crown Use (Seale, 2002; American Academy of Pediatric Dentistry, 2020; Dean, Avery, & McDonald, 2016)

- Primary teeth with more than half of their root resorption completed
- Primary teeth nearing natural exfoliation (approximately 6–12 months remaining)
- Teeth exhibiting marked mobility
- Patients with nickel allergy or sensitivity
- Inability to cement the crown due to lack of cooperation

Steps Before Tooth Preparation and Cementation Crown Selection

- Measuring the mesiodistal width of the tooth before preparation
- Trying different crown sizes using a trial-and-error approach to determine the best fit
- Choosing the crown that provides the best adaptation after tooth preparation (Seale & Randall, 2015; McDonald, Avery, & Dean, 2016)

Occlusal Assessment

- Evaluating eruption status of the opposing tooth, especially when delayed due to caries
- Assessing whether mesial drift has occurred due to loss of proximal contact from caries
- Analyzing the occlusal relationships of incisors, canines, and molars on both sides using intraoral examination or dental casts
- Carefully examining the midline relationship and cusp-fossa relationships bilaterally (Dean, Avery, & McDonald, 2016; Pinkham, Casamassimo, Fields, McTigue, & Nowak, 2013)

Local Anesthesia Rubber Dam Isolation

Rubber dam use is recommended to:

- Prevent injury to adjacent tissues
- Improve visibility and clinical efficiency
- Facilitate behavior management in children
- Eliminate the risk of accidental swallowing of the crown

(Seale, 2002; Pinkham, Casamassimo, Fields, McTigue, & Nowak, 2013; American Academy of Pediatric Dentistry, 2020)

Wedge Placement

Many researchers have recommended wedge placement before preparation. Wedges help create slight separation between adjacent teeth, reducing the risk of iatrogenic enamel damage.

(Seale & Randall, 2015; McDonald, Avery, & Dean, 2016)

Removal of Caries

Caries removal and pulpotomy procedures may be performed either before or after crown preparation; however, in many cases, the preparation required for crown placement is naturally achieved during caries excavation (Dean, Avery, & McDonald, 2016; Pinkham, Casamassimo, Fields, McTigue, & Nowak, 2013).

Technique / Treatment

During caries removal and shaping of proximal surfaces, the optimal approach is to begin with occlusal reduction (Seale & Randall, 2015; Dean, Avery, & McDonald, 2016).

Occlusal Reduction

Preserving the natural morphology of the cusps is essential. Depth-orientation grooves may be placed on the occlusal surface to achieve more controlled reduction. If significant occlusal structure has already been lost due to caries, the

marginal ridges of adjacent teeth can serve as reference points. Generally, 1.5–2 mm of occlusal reduction is sufficient (Seale & Randall, 2015; Dean, Avery, & McDonald, 2016; Pinkham, Casamassimo, Fields, McTigue, & Nowak, 2013).

Proximal Reduction

The mesial and distal contact areas should be opened enough to allow passage of a probe. Following the natural proximal contour, these surfaces should taper slightly toward the occlusal and lingual directions. A smooth taper from the occlusal toward the gingiva must be established without creating ledges or shoulders, and no undercuts should remain (Seale & Randall, 2015; Dean, Avery, & McDonald, 2016; Pinkham, Casamassimo, Fields, McTigue, & Nowak, 2013).

Buccal/Lingual Reduction

Reduction of the buccal and lingual surfaces constitutes the third step of preparation. Some researchers recommend trying in the selected crown before preparing these surfaces.

In the final stage, all line angles and point angles should be softened and rounded (Seale & Randall, 2015; Dean, Avery, & McDonald, 2016; Pinkham, Casamassimo, Fields, McTigue, & Nowak, 2013).

Crown Adaptation

Proper crown adaptation is essential for retention and periodontal health. A poorly adapted crown can lead to plaque accumulation, gingival inflammation, and cervical recurrent caries (Seale & Randall, 2015; Dean, Avery, & McDonald, 2016; Pinkham, Casamassimo, Fields, McTigue, & Nowak, 2013).

Cementation

An appropriate luting cement must be used for crown placement. Once fully seated, the crown should be maintained under pressure until the cement sets. Manufacturers recommend the use of glass ionomer, zinc polycarboxylate, or zinc phosphate cements; additionally,

fluoride-releasing cements offer extra protective benefits. Due to its clinical advantages, resin-modified glass ionomer cement (RMGIC) is generally considered the first-choice material. However, studies show that the type of cement has minimal influence on overall retention; the primary determinants are proper crown contouring and adequate seating pressure (Seale & Randall, 2015; Dean, Avery, & McDonald, 2016; Croll & Nicholson, 2002).

Complications Interproximal Overhang

If undercuts are not eliminated, difficulties may arise during crown placement (Seale & Randall, 2015; Dean, Avery, & McDonald, 2016).

Marginal Discrepancies

Numerous studies have shown a strong relationship between gingivitis and marginal defects such as poor crown adaptation or incomplete removal of excess

cement. Therefore, careful marginal fitting before placement and meticulous finishing and polishing afterward play crucial roles in reducing gingivitis risk (Seale & Randall, 2015; Dean, Avery, & McDonald, 2016; Croll, 1999).

Crown Aspiration or Swallowing

To prevent crown aspiration, rubber dam isolation is recommended until the cementation stage. In some cases, the dam may need to be removed before preparing the distal surface (Pinkham, Casamassimo, Fields, McTigue, & Nowak, 2013; American Academy of Pediatric Dentistry, 2020; Seale & Randall, 2015).

Clinical Significance of Stainless Steel Crowns

Primary teeth exhibit a higher susceptibility to caries than permanent teeth. Their pulp chambers are larger and pulp horns more prominent, requiring special care during cavity preparation. Because of these anatomical features, full-coverage restorations are frequently necessary (Seale, 2002; Pinkham, Casamassimo, Fields, McTigue, & Nowak, 2013; Dean, Avery, & McDonald, 2016).

Despite some esthetic limitations, stainless steel crowns remain a widely preferred treatment option due to their:

1. High durability
2. Strong protective capacity
3. Long-term success
4. Cost-effectiveness
5. Reliable clinical outcomes

(Seale, 2002; Seale & Randall, 2015; American Academy of Pediatric Dentistry, 2020)

Clinical Evaluation of Zirconia and Stainless Steel Crowns for Pediatric Posterior Teeth: A Comparative Study

In a survey conducted among pediatric dentists, 87% reported that parents were concerned about the esthetic appearance of restorations placed on posterior teeth. Studies also show that esthetics is important for children as well, influencing their psychological well-being and self-perception (Pani et al., 2016; Fishman, Guelmann, & Bimstein, 2007).

Among the advantages of prefabricated zirconia crowns are their high esthetic performance, the absence of additional components that may debond, and lower technique sensitivity compared with other esthetic alternatives. Disadvantages include the need for increased removal of tooth structure, the inability to compress or contour the crown, and higher overall cost (Clark et al., 2016).

Twenty-nine children aged 3–9 years were included in the study, and a total of 70 prefabricated crowns were placed (35 zirconia crowns and 35 stainless steel crowns). The crowns were evaluated at 3, 6, 9, and 12 months for retention,

marginal adaptation, wear on opposing teeth, plaque accumulation, gingival inflammation, and proximal contacts (Murali, Mungara, Vijayakumar, & Patil, 2022).

By the end of the follow-up period, the overall success rate was 93.5% for zirconia crowns and 96.7% for stainless steel crowns. Statistical analysis revealed no significant difference between the two groups (Murali, Mungara, Vijayakumar, & Patil, 2022).

Both prefabricated zirconia crowns and stainless steel crowns demonstrated successful clinical outcomes for posterior teeth. When esthetics is a primary consideration, prefabricated zirconia crowns may be regarded as a suitable alternative for full-coverage restorations in posterior teeth (Murali, Mungara, Vijayakumar, & Patil, 2022).



Figure 1.4.1. Stainless steel crowns on maxillary molars (Murali vd., 2022)



Figure 1.4.2. Prefabricated zirconia crowns on mandibular second molars (Murali vd., 2022)



Figure 1.4.3. In occlusion (Murali vd., 2022)

1.5. CAD/CAM

Applications of Intraoral Scanners and CAD/CAM Systems in Pediatric Dentistry

Intraoral Scanners and Their Use in Pediatric Patients

With the increasing adoption of digital methods in the impression-taking process in dentistry, a significant transformation has taken place. As these technologies evolve, discomfort associated with conventional impressions decreases in both adults and children, making the procedure far more tolerable (Hacker, Heydecke, & Reissmann, 2015).

Because treatment durations in children must generally be shorter than in adults, various challenges may arise when applying conventional impression techniques (Oba, Dulgergil, & Sonmez, 2009).

In a study comparing digital and conventional impression methods in children with respect to comfort, preference, and impression-taking time, digital impressions were found to be easier, more comfortable, and more readily accepted by pediatric patients (Gjelvold, Chrcanovic, Korduner, Collin-Bagewitz, & Kisch, 2016).

Use of CAD/CAM Systems in Restorations for Pediatric Patients

CAD/CAM-fabricated restorations have become a widely used treatment option for permanent teeth in children. A recent case report described a CAD/CAM-fabricated full crown placed on a permanent first molar of an eight-year-old patient following root canal treatment. The restoration successfully met the child's esthetic and functional expectations and maintained marginal integrity and structural continuity (Davidovich, Shay, Mijiritsky, & Nuni, 2020).

CAD/CAM-fabricated endocrowns represent an effective and well-adapted restorative alternative in pediatric dentistry for endodontically treated teeth. They provide a conservative approach while offering high compatibility with the remaining tooth structure (Einhorn DuVall, Wajdowicz, Brewster, & Roberts, 2019; Bilgin, Erdem, & Tanriver, 2016).

Studies have reported that CAD/CAM restorations applied to primary teeth with extensive structural loss provide both esthetically satisfying and functionally successful outcomes (Demirel, Bezgin, Akaltan, & Sarı, 2017; Mourouzis, Arhakis, & Tolidis, 2019).

Digital Dentistry Applications in Pediatric Dentistry

(Gülbahçe, Berber, & Yetkiner, 2022)

Space Maintainers

One of the innovations that has emerged with the expansion of digital applications in pediatric dentistry is the digital fabrication of space maintainers used after premature loss of primary teeth. These appliances—produced using CAD/CAM systems or 3D printers—are prepared with modern, biocompatible

materials and are referred to as digital space maintainers (Dhanotra & Bhatia, 2021).

Because traditional space maintainers can corrode or fracture easily, esthetic, metal-free alternatives have been developed. These include fiber-reinforced composite space maintainers and ceramic band-and-loop maintainers produced using CAD/CAM technology (Soni, 2017).

Steps of Digital Space Maintainer Production Using CAD/CAM:

1. Digital acquisition of the intraoral scan or digitization of a model obtained from a conventional impression
2. Creation of a virtual model using these data
3. Designing the space maintainer in CAD software by defining abutment teeth, retentive areas, and cementation zones
4. Transferring the final design to a CAM system and milling the chosen material to fabricate the appliance

(Ierardo, Luzzi, Lesti, Sfasciotti, & Polimeni, 2017)

Clinical Evaluation of Personalized 3-D Printed and CAD/CAM Resin Crowns as Alternatives to Stainless Steel Crowns in Pediatric Dentistry

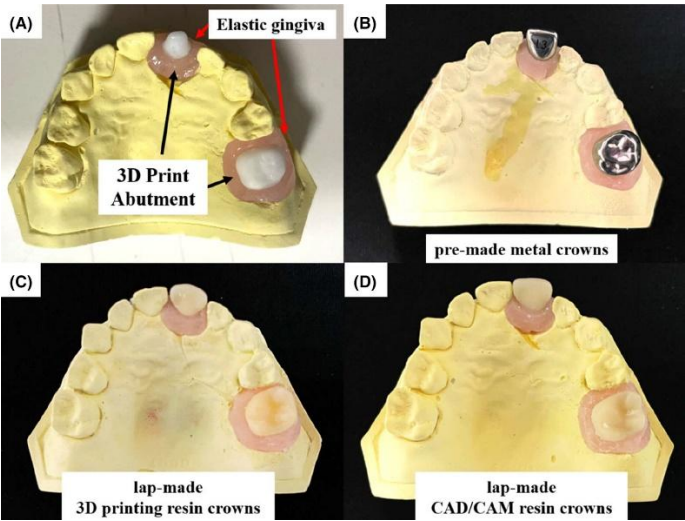


Figure 1.5.1. Model and crowns for this study. (A) Working models. (B–D) Pediatric dental crowns fabricated in different forms mounted on the model. (Huang et al., 2024)

Children with dental caries can be treated using stainless steel crowns (SSCs), yet the need to improve esthetics and function persists. Today, both 3D-printed resin crowns (PRCs) and CAD/CAM-fabricated resin crowns (CRCs) have emerged as options that meet clinical expectations for teeth requiring full-

coverage restorations in terms of durability, production time, cost, and esthetics (Huang et al., 2024).

The characteristics of 3D-printed crowns can be summarized as follows:

- Fabricated using an additive manufacturing technique
- Resin is light-cured layer by layer to form the structure
- SLA or DLP-based technologies are used
- Material waste during production is minimal

CAD/CAM crowns have the following characteristics:

- Manufactured using a subtractive milling technique
- A pre-polymerized block is milled into the desired shape
- CNC milling machines are used in fabrication
- A more homogeneous and dense internal structure is achieved

(Miyazaki et al., 2009; Zimmermann et al., 2015; Ting-Shu & Jian, 2015)

In the study, a total of 60 crowns were prepared—10 PRCs, 10 CRCs, and 10 SSCs each for incisors and molars. Production efficiency, surface properties, marginal fit, and stability of the two resin crown types were compared with SSCs. Although PRCs and CRCs demonstrated superior color matching and surface characteristics compared with SSCs, their production times were significantly longer (5.3–12.4 times longer for PRCs; 3.3–9.1 times longer for CRCs) ($p < 0.05$). Nevertheless, both types of crowns could still be completed in approximately 80 minutes. In addition, the marginal gaps of PRCs and CRCs were found to be considerably lower than those of SSCs (13.0–19.2 times and 13.0–13.7 times lower, respectively) ($p < 0.05$). All materials generally exhibited good overall stability (Huang et al., 2024).

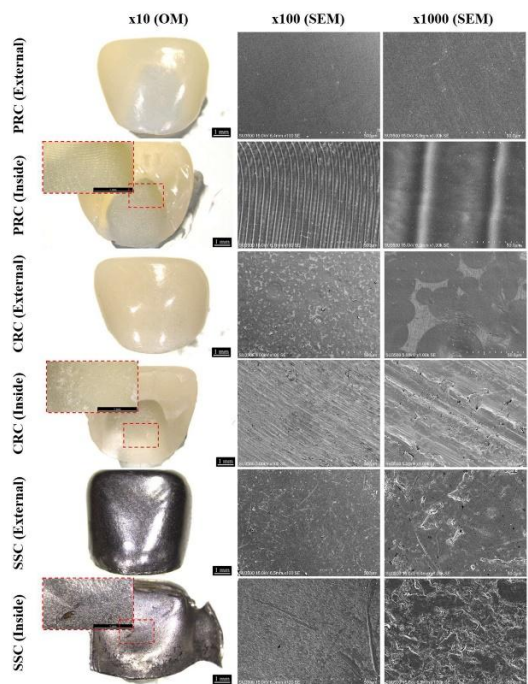


Figure 1.5.2. Surface topography of 3D-printed resin crowns, CAD/CAM resin crowns, and stainless steel crowns. (Huang et al., 2024)

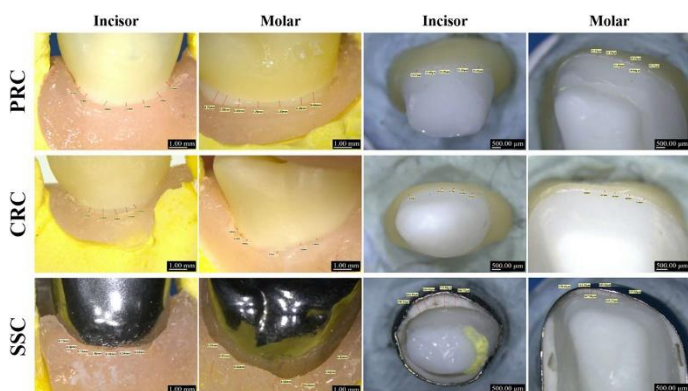


Figure 1.5.3. Measurement of the distance between the crown and the abutment teeth. (Huang et al., 2024)

An Innovative Treatment Approach Using a Digital Workflow and CAD/CAM Section 1: Restoration of Endodontically Treated Molars in Children

Stainless steel crowns (SSCs) remain the most commonly preferred method for restoring young permanent first molars that have undergone endodontic treatment

in children.

According to the American Academy of Pediatric Dentistry, metal crowns are indicated for the treatment of permanent molars in children with extensive caries or developmental defects, in cases where existing restorations have failed, and for teeth that have received root canal treatment (American Academy of Pediatric Dentistry, 2020).

SSCs provide effective protection against future caries due to their full-coverage design and high durability (Seale, 2002).

Some disadvantages of SSCs have led to the development of alternative methods such as endocrowns. Endocrowns have been shown to be a reliable restorative option for severely damaged molars and premolars, even in the presence of extensive coronal tissue loss or risk factors such as bruxism and unfavorable occlusal relationships. Systematic reviews report high success rates ranging from 94% to 100%, and endocrowns demonstrate greater fracture resistance compared with conventional restorative approaches (Belleflamme et al., 2017).

In children and in individuals with a pronounced gag reflex, intraoral scanners allow impressions to be obtained rapidly without the need for impression materials or trays. Studies have shown that such scanners can achieve accuracy comparable to traditional methods for single-tooth restorations and short-span bridges (Koch & García-Godoy, 2000; Means & Flenniken, 1970).

Intraoral scanners are generally more acceptable to pediatric patients and easier to apply clinically. However, before use, the child's behavioral status, parental cooperation, and preparatory needs must be carefully considered, along with factors such as inhalation sedation, effective local anesthesia, and rubber dam isolation (Davidovich, Shay, Nuni, & Mijiritsky, 2020).

Creating a 1–2 mm ferrule is a critical step in endocrown preparation, and the tooth must be shaped appropriately for endocrown placement in both premolars and molars (Davidovich, Shay, Nuni, & Mijiritsky, 2020).

For endocrowns fabricated using intraoral scanners, the involved tooth and the opposing arch must be digitally scanned. Once the restoration is designed and produced using a CAD/CAM system, the endocrown is cemented appropriately and monitored through regular follow-up appointments (Davidovich, Shay, Nuni, & Mijiritsky, 2020).



Figure 1.5.4. Radiographic image taken after completion of root canal treatment on the maxillary right first molar. (Davidovich, Shay, Nuni, & Mijiritsky, 2020)



Figure 1.5.5. Scan of tooth 16 before tooth preparation. (Davidovich, Shay, vd., 2020)



Figure 1.5.6. Scanning image of tooth 16 after tooth preparation. (Davidovich, Shay, vd., 2020)



Figure 1.5.7. Scan of the opposing arch.(Davidovich, Shay, vd., 2020)



Figure 1.5.8. Cementation of the restoration.(Davidovich, Shay, vd., 2020)

An Innovative Treatment Approach Using a Digital Workflow and CAD/CAM Section 2: Restoration of Molar–Incisor Hypomineralization in Children

Until recently, the treatment of molar–incisor hypomineralization (MIH) primarily relied on resin restorations and temporary options such as stainless steel crowns (SSCs), which typically required replacement after adolescence. With the introduction of intraoral scanners, a new treatment option has emerged that facilitates the clinical process in uncooperative children, preserves tooth structure more effectively, and enhances restoration longevity (Davidovich, Dagon, Tamari, Etinger, & Mijiritsky, 2020).

Intraoral scanners allow impressions to be obtained in a very short time and eliminate the need for impression materials or trays. This feature provides a considerable advantage for pediatric patients and individuals with a pronounced gag reflex (Almeida et al., 2014).

Furthermore, intraoral scanners have been shown to provide accuracy comparable to traditional impression techniques for single-tooth restorations and short-span bridges (Almeida et al., 2014; Tsirogiannis, Reissmann, & Heydecke, 2016).

Optical impressions taken with IOS can help overcome difficulties encountered in conventional impression techniques, especially in technically complex cases (Ting-Shu & Jian, 2015; Zimmermann, Mehl, Mörmann, & Reich, 2015; Almeida et al., 2014).

At the beginning of treatment, inhalation sedation, effective local anesthesia, and rubber dam isolation may be used when necessary. All porous areas in enamel and dentin must be removed. In molars, inlay or onlay restorations are generally preferred, whereas labial veneers are used for incisors. The teeth are prepared accordingly to accommodate these indirect restorations. IOS-based impression

taking involves scanning the prepared tooth, the opposing arch, and the occlusion, followed by CAD/CAM fabrication of the restoration. The final step includes cementation of the restoration and routine follow-up. Due to the high scanning accuracy of the digital workflow, restorations can be fabricated with almost no errors (Davidovich, Dagon, Tamari, Etinger, & Mijiritsky, 2020).



Figure 1.5.9. Scan of tooth 36 before tooth preparation. (Davidovich, Dagon, Tamari, Etinger, & Mijiritsky, 2020)



Figure 1.5.10. Scanning image of tooth 36 after tooth preparation. (Davidovich, Dagon, vd., 2020)



Figure 1.5.11. The fabricated crown restoration before cementation. (Davidovich, Dagon, vd., 2020)



Figure 1.5.12. Cementation of the restoration. (Davidovich, Dagon, vd., 2020)

1.6. Zirconium crowns

Dental caries is defined as a multifactorial, chronic infectious process affecting the mineralized structures of the tooth, characterized by mineral loss and degradation of organic tissue, ultimately resulting in cavity formation (Shafer, Hine, & Levy, 2012).

The clinical presentation of caries extends beyond physical discomfort; it also carries psychosocial consequences that significantly impact the patient's quality of life. This situation may lead to a notable decline in academic performance, in addition to emotional difficulties such as diminished self-esteem, social withdrawal, and anxiety disorders (Thomas & Primosch, 2002).

Restorative Treatment Options and Material Selection

In cases where caries affects multiple surfaces or compromises the structural integrity of the tooth, crown restorations emerge as an indispensable treatment alternative. While conventional filling materials carry a risk of fracture under functional forces and may weaken the remaining tooth structure, prefabricated crowns compensate for these structural inadequacies, providing a more durable restorative option (Schüler, Hiller, Roloff, Kühnisch, & Heinrich-Weltzien, 2014).

Stainless steel crowns, introduced to the literature by Humphrey, remain widely used owing to their strength, practical applicability, and economic advantages. However, their metallic gray appearance is incompatible with contemporary aesthetic expectations, limiting their use particularly in visible regions and directing clinicians and families toward more natural-appearing alternatives (Roberts, Lee, & Wright, 2003).

Zirconium crowns, an aesthetically oriented option, are notable for their robust mechanical properties and excellent tissue compatibility. They are chemically inert and resistant to corrosion; their smooth surface characteristics minimize plaque accumulation and the accompanying gingival inflammation (Waggoner & Cohen, 2010).

Nevertheless, this material also presents certain technical limitations. Despite its high fracture resistance, the meticulous procedures required during preparation, relatively prolonged clinical application time, and elevated cost are considered disadvantages of zirconium (Alaki, Abdulhadi, & AbdElBaki, 2020).

Periodontal Tissue Health and Material Compatibility

Clinical data demonstrate that zirconium restorations exhibit less plaque accumulation and minimal gingival inflammation. The foundation of this biological superiority lies in the highly polished, non-porous surface structure that impedes bacterial adhesion (Taran & Kaya, 2018; Aggarwal, Goyel, Mathur, & Sachdev, 2022).

The biological benefits offered by zirconium crowns are related not only to the physicochemical properties of the material but also to its design features. These crowns, with their rounded contours reflecting natural tooth morphology, support a "self-cleansing" mechanism and reduce the accumulation of food debris (Skjold, Schriwer, & Øilo, 2019).

In conclusion, zirconium crowns represent an important alternative for maintaining periodontal health in both anterior and posterior regions by combining aesthetic superiority with tissue-friendly surface characteristics (Alrassan et al., 2025).

Resistance to Discoloration and Aesthetic Longevity

In the pediatric population, long-term clinical success depends not only on structural durability but also directly on color stability, a fundamental component of visual integrity. Zirconium crowns exhibit a distinctly more resistant character against staining when compared with other crown types. The source of this advantage is the material's microstructure obtained through intensive sintering; this characteristic minimizes surface porosity, thereby preventing pigment accumulation from dietary sources and limiting plaque formation that could compromise aesthetic appearance (Almajed, 2024).

Although zirconium crowns demonstrate high resistance to color change, it should be noted that they are not entirely impervious. Laboratory studies indicate that prolonged exposure to acidic and highly pigmented liquids may result in

slight color differences; however, these changes typically remain below the clinical threshold detectable by the human eye (Abdou, Mohamady, Mahmoud, & Abo-Elhoud, 2025). While these findings support the relative superiority of zirconium, they also emphasize that long-term aesthetic success must be reinforced by individual dietary habits and oral hygiene practices.

Another strength of zirconium is its optical compatibility. Owing to its light-reflecting capacity and translucent structure, it successfully mimics the natural tooth appearance. This optical performance provides a natural aesthetic particularly in anterior restorations while contributing to the crown maintaining an acceptable appearance despite the slight color changes that adjacent teeth may undergo over time (Pereira et al., 2021).

1.7. Fixed space maintainers

Early loss of primary teeth not only leads to impairment of masticatory function but also significantly increases the likelihood of malocclusion by adversely affecting the eruption path of permanent teeth due to the spaces created within the dental arch (Rodrigues, Dourado, de Araújo, No-Cortes, & Pinhata-Baptista, 2022).

In cases of unilateral single primary molar loss, band/crown-loop space maintainers are classically adopted as the standard treatment approach. However, the fabrication process of these appliances entails notable cooperation difficulties, particularly in pediatric patients.

Conventional alginate impression materials may negatively affect the patient's treatment experience by causing discomfort such as strong gag reflex and breathing difficulties (Tokuc & Yilmaz, 2022). From a clinical perspective, mechanical problems including band fractures, cementation failure, solder joint separation, and impingement of the loop component into gingival tissues threaten the long-term success of these appliances (Lee, 2022). Furthermore, their inability to meet aesthetic requirements due to their metallic appearance and their limited contribution to masticatory function have made the investigation of alternative options inevitable (Soni, 2017; Ge, 2020).

Within this framework, intraoral digital scanners and CAD/CAM (Computer-Aided Design and Manufacturing) technologies mark the beginning of an innovative era in pediatric dentistry. Polyetheretherketone (PEEK), one of the prominent materials of this technological advancement, is an engineering polymer distinguished by its high mechanical durability, resistance to corrosion, and superior biocompatibility properties (Hallab, McAllister, Brady, & Jarman-Smith, 2012). Currently employed successfully in the fabrication of fixed and removable partial prostheses, PEEK provides a biomechanical advantage owing to its modulus of elasticity being close to that of bone tissue (Guo, Wang, Zhao, & Liu, 2020; Harb, Abdel-Khalek, & Hegazy, 2019). Its capacity to be processed with micron-level precision through CAD/CAM systems strengthens PEEK's position in digital dentistry applications.



Figure 1.7.1. Preoperative view (1a mandibular occlusal view; 1b lateral view) (Wang et al., 2023).

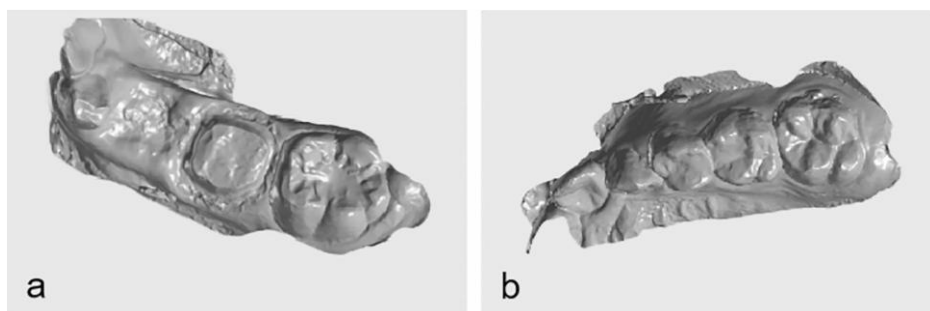


Figure 1.7.2. Digital model (2a mandibular model; 2b maxillary model) (Wang et al., 2023).

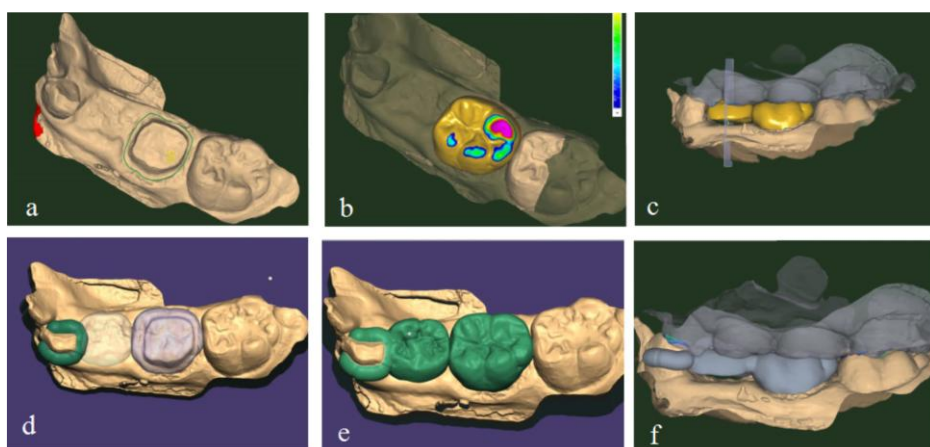


Figure 1.7.3. Digital design of the semi-rigid bridge space maintainer (full crown) (3a determined margin line; 3b full crown design; 3c pontic design; 3d non-rigid connector retainer design; 3e connector design; 3f verification of occlusal relationship) (Wang et al., 2023).

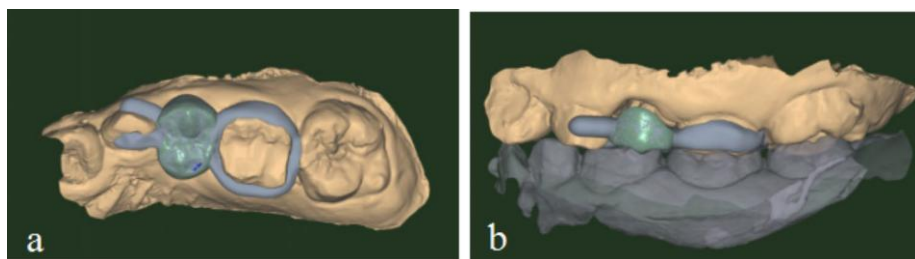


Figure 1.7.4. Digital design of the semi-rigid bridge space maintainer (band type) (Wang et al., 2023).



Figure 1.7.5. PEEK Digital Semi-Rigid Bridge Space Maintainer (Wang et al., 2023).

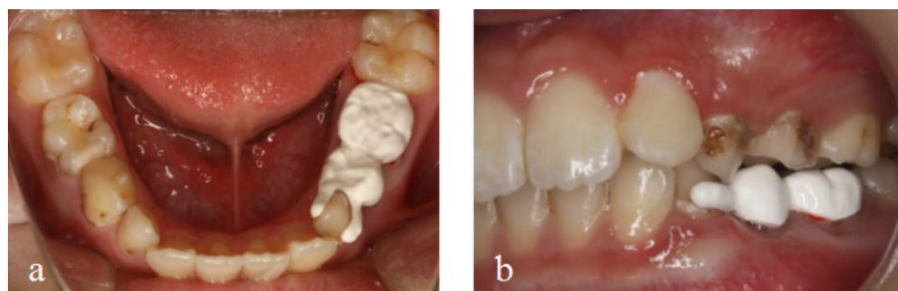


Figure 1.7.6. View of the PEEK semi-rigid bridge space maintainer (6a mandibular occlusal view; 6b lateral view) (Wang et al., 2023).

Currently, band-loop or crown-loop space maintainers are widely preferred as the gold standard in cases of early loss of a single primary molar. However, the fabrication protocol of conventional space maintainers requires a considerably complex and multistage process.

The success of a classical space maintainer is largely dependent on the dimensional stability of the impression material and the manual skill of the dental technician; this situation increases the probability of human-induced error at each stage, thereby jeopardizing the fit quality of the appliance. Another significant limitation from a clinical application standpoint is the unavailability of bands with appropriate anatomical form for first primary molars under all circumstances. This situation may necessitate the preparation of sound adjacent teeth in crown-loop applications, leading to an invasive approach.

From a functional perspective, conventional space maintainers cannot fully restore masticatory function as they do not provide occlusal support. More importantly, since they focus solely on preserving horizontal distance, they remain inadequate in maintaining vertical space and preventing supraeruption of teeth in the opposing arch (Wang et al., 2023).

LINGUAL ARCH

Primary teeth play a fundamental role in the healthy maintenance of speech and masticatory functions, in addition to preserving their natural positions within the dental arch until the permanent teeth erupt (Mittal, Sharma, Sharma, Gupta, Gaur, & Pathania, 2018). The literature indicates that the most ideal "space maintainer" for permanent teeth is the primary teeth themselves. However, in the event of early loss of these teeth, the use of orthodontic appliances becomes mandatory to maintain arch integrity and prevent potential space loss (Boo Gordillo, Marqués Martínez, Borrell García, & García Miralles, 2024).

The mandibular lingual arch is an effective space maintainer widely recommended to preserve mandibular arch length, limit mesial migration of first permanent molars, and prevent arch narrowing by restricting lingual tipping of incisors (Laing, Ashley, Naini, & Gill, 2009). The fundamental biomechanical advantage offered by the lingual arch is that it holds the mandibular incisors stable in their existing positions while halting mesial movement of molars through a physical barrier (Owais, Rousan, Badran, & Abu Alhaija, 2011).

One of the most significant advantages provided by 3D printing (additive manufacturing) technology is that it enables the appliance to be fabricated monolithically (as a single piece). This structural integrity minimizes the risk of fracture since it contains no solder joints and maintains structural durability even under strong occlusal forces. The digital workflow standardizes manual laboratory stages, thereby minimizing human error and significantly reducing production time (Pawar, 2019).



Figure 1. Intraoral periapical radiograph of the mandibular left posterior region showing extensive cervical caries on teeth 74 and 75 (Trivedi, Aduri, Khan, & Pande, 2024).

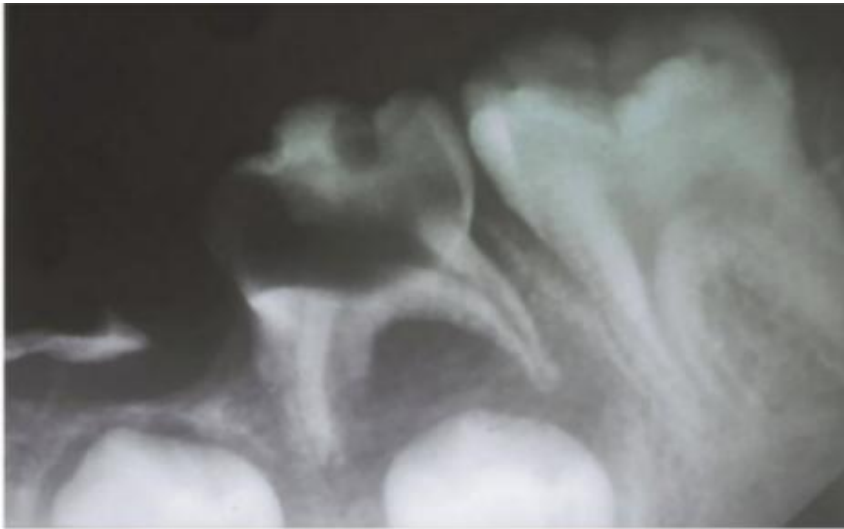


Figure 2. Intraoral periapical radiograph of the mandibular right posterior region showing root remnants of tooth 84 and complete fracture of the mesial and lingual walls of tooth 85 (Trivedi et al., 2024).

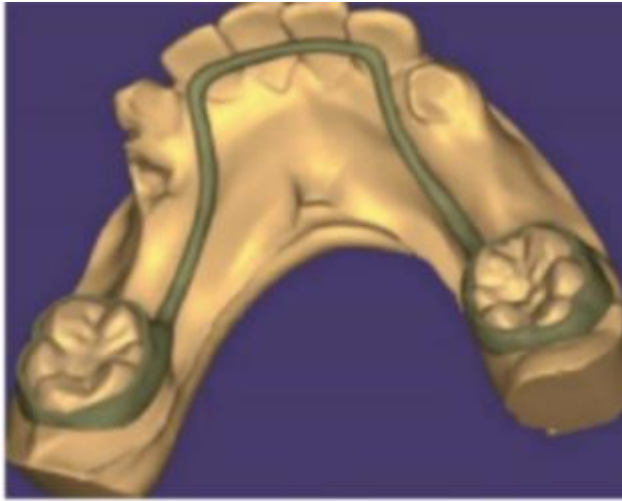


Figure 3. Design of the 3D Lingual Arch Space Maintainer (Trivedi et al., 2024).



Figure 4. Postoperative occlusal view of the mandible following placement of the 3D-printed lingual space maintainer (Trivedi et al., 2024).

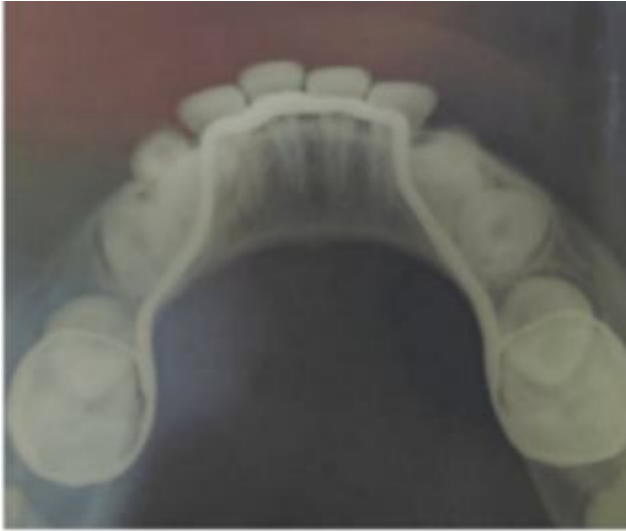


Figure 5. Radiographic occlusal view of the mandible showing the 3D-printed lingual arch space maintainer (Trivedi et al., 2024).

2. REMOVABLE PROSTHESES

2.1. Removable Space Maintainers

Early loss of primary teeth can disrupt dental arch integrity, adversely affecting the eruption path and ideal occlusal alignment of permanent teeth (Park, Jung, & Kim, 2009). For this reason, the use of space maintainers to preserve arch length and guide occlusion within physiological limits is accepted as a standard procedure in pediatric dentistry practice (Qudeimat & Sasa, 2015; Ahmad, Parekh, & Ashley, 2018). Particularly in cases where multiple primary molars are lost consecutively, removable space maintainers are preferred to provide functional improvement (Law, 2013).

The principal clinical benefits provided by removable space maintainers are as follows:

- **Dimensional Preservation:** Maintenance of mesial, distal, and proximal distances
- **Vertical Stabilization:** Preservation of occlusal height and prevention of supraeruption of teeth in the opposing arch
- **Aesthetic Improvement:** Restoration of dental aesthetics and support of the patient's psychosocial development
- **Phonetic Support:** Prevention of speech problems associated with early tooth loss
- **Functional Balance:** Prevention of pathological habits such as unilateral mastication (Goenka, Sarawgi, Marwah, Gumber, & Dutta,

2014; Mohammad, Cheruku, Penmetcha, Bagalkotkar, & Kumari, 2015; Kalia, Tandon, Bhupali, Rathore, Mathur, & Rathore, 2018)

However, conventional removable space maintainer fabrication entails significant limitations during clinical and laboratory stages (Srivastava, Grover, & Panthri, 2016). The classical method may yield results that are difficult to standardize due to its requirement for technical precision and high dependence on technician experience. Manual bending of clasps and the use of auto-polymerizing (self-cure) acrylic resin further complicate the process. Dimensional shrinkage occurring particularly during polymerization can cause irritation by compromising the fit of the appliance with the mucosa and abutment teeth (Kawara, Komiyama, Kimoto, Kobayashi, Kobayashi, & Nemoto, 1998). This misfit significantly reduces comfort and treatment compliance in pediatric patients. Furthermore, modified permanent tooth forms used due to the unavailability of artificial primary tooth forms in appropriate sizes and shapes in the market cannot fully replicate primary tooth anatomy. All these factors clearly demonstrate the need for more precise, rapid, and predictable fabrication methods.

The combination of CAD/CAM-assisted digital design with PEEK (Polyetheretherketone) material offers an innovative solution that eliminates the chronic disadvantages of conventional removable space maintainers. This technological integration provides micron-level precise fit free from manual fabrication errors, complete reproducibility through digital data, significant time savings in laboratory and clinical chairside procedures, and enhanced patient comfort through biomechanical compatibility.

Particularly in pediatric cases where patient cooperation is limited and aesthetic expectations are high, PEEK's natural appearance approximating tooth color and its lightweight structure constitute a critical advantage that directly enhances treatment success and patient compliance (Trivedi et al., 2024).

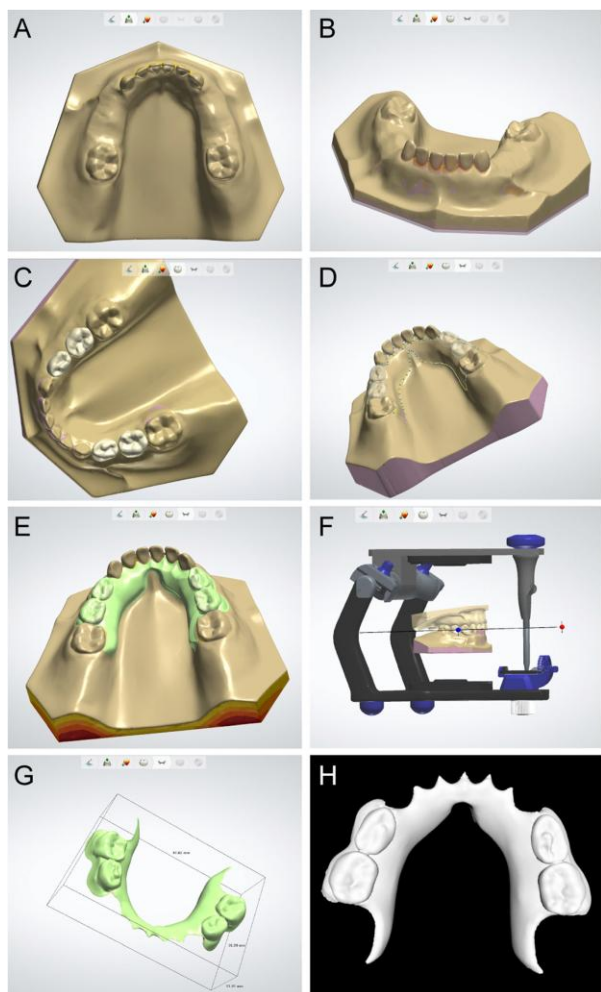


Figure 1. Digital design process of removable space maintainers; a) import of model data; b) inspection of the model and filling of undercuts/voids; c) import of relevant models from the artificial primary tooth database (DIY) into the software; d) creation of the major connector profile; e) assembly of the integrated removable space maintainer; f) occlusal adjustment using a virtual articulator; g) final removable space maintainer; and h) final removable space maintainer data exported in STL format (Trivedi et al., 2024).

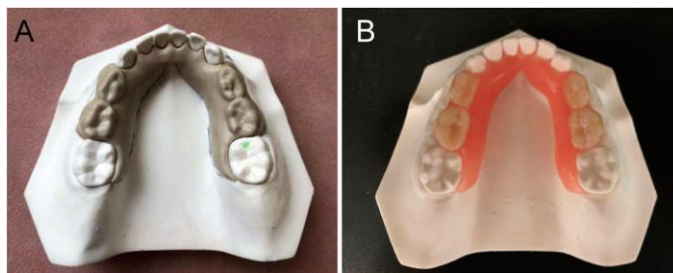


Figure 2. a) Polyetheretherketone (PEEK) removable space maintainer; **b)** Laboratory fabrication of removable space maintainers (Trivedi et al., 2024).

2.2. Complete dentures

Prosthetic treatment modalities play a vital role in the rehabilitation of children whose dentofacial development has been adversely affected. The underlying cause of complete or partial denture requirements at early ages is most commonly the congenital absence of teeth (Tarjan, Gabris, & Rozsa, 2005). Hereditary conditions such as Hypohidrotic Ectodermal Dysplasia and Papillon-Lefèvre syndrome may result in severe clinical presentations characterized by oligodontia or anodontia. Beyond this, early loss of primary teeth as a consequence of uncontrollable extensive caries processes emerges as an acquired factor necessitating complete denture application even in preschool-aged children (Paul, Tandon, & Kiran, 1995).

The management of oligodontia cases constitutes a complex process for clinicians not only due to the technical difficulties posed by the absence of numerous teeth but also because of the considerably young age at which patients require treatment (Mussa, Esposito, & Cowper, 1999). Although various strategies for treating anodontia, oligodontia, and morphological anomalies have been described in the literature, conventional removable complete or partial dentures continue to remain the most frequently preferred restorative option in this young patient group undergoing growth and development (Mussa et al., 1999; Pigno, Blackman, Cronin, & Cavazos, 1996; Vieira, Teixeira, Guirado, & Gavião, 2007; Pettit & Campbell, 2010).

The growth and preservation of alveolar bone tissue is directly dependent on the osteogenic stimulus provided by the presence of teeth. Consequently, alveolar ridge atrophy becomes an inevitable condition in children with oligodontia or anodontia; this situation leads to inadequacy of the bony foundation necessary for denture support and retention (Paschos, Huth, & Hickel, 2002; Vieira et al., 2007). The edentulous condition may result in multidimensional systemic consequences including speech disorders, atypical behavioral patterns developing in the tongue, and nutritional deficiencies related to impaired masticatory function (Al-Ibrahim, Al-Hadlaq, Abduljabbar, Al-Hamdan, & Abdin, 2012; Kohli, Levy, Kummet, Dawson, & Stanford, 2011). In addition to all these clinical findings, the profound negative effects of aesthetic concerns arising from tooth loss on the

child's self-esteem render the psychosocial dimension of the rehabilitation process an important area that the clinician must separately address (Al-Ibrahim et al., 2012; Vieira et al., 2007).



Figure 1. Maxillary and mandibular silicone impressions (Teixeira Marques et al., 2013).



Figure 2. Initial frontal view of the patient. Reduced vertical dimension is noteworthy (Teixeira Marques et al., 2013).



Figure 3. Working casts mounted on a simple articulator (Teixeira Marques et al., 2013).



Figure 4. Frontal view of working casts mounted on a simple articulator and artificial tooth arrangement (Teixeira Marques et al., 2013).



Figure 5. Wax try-in of maxillary and mandibular complete dentures (Teixeira Marques et al., 2013).



Figure 6. Artificial teeth processed in acrylic resin (Teixeira Marques et al., 2013).



Figure 7. Maxillary and mandibular complete dentures in occlusion (Teixeira Marques et al., 2013).

2.3. Complete Dentures Indications for Overdentures:

Early loss of primary teeth in children typically results from advanced dental caries (early childhood caries) or trauma (such as falls or impacts). In such cases, the aim of treatment is to restore the child's chewing, speech, and aesthetic functions, and to prevent space loss in areas where permanent teeth will erupt (McDonald, R. E., Avery, D. R., & Dean, J. A., 2016).

Congenital absence of teeth

In some children, for example in conditions such as ectodermal dysplasia, teeth may fail to develop entirely. Overdentures used in these situations help meet functional needs at a young age while also providing an improved aesthetic appearance (Gupta, Goel, & Verma, 2012).

Diseases associated with defective tooth structure

Although teeth are present, some children may have extremely weak, very small, or easily worn-down teeth. Conditions such as amelogenesis imperfecta and dentinogenesis imperfecta commonly present with this pattern. In these cases, the dental roots are preserved, and appropriate prostheses are fabricated over them (Crawford, Aldred, & Bloch-Zupan, 2007).

Teeth with destroyed crowns but intact roots

Even when the crown portion is severely damaged, if the root remains healthy, the tooth is not extracted; the root is retained in the mouth, and an overdenture is constructed over it. This approach contributes to the preservation of alveolar bone volume and health (Preiskel, H. W., 1996).

Supporting growth and development

Overdentures may also be used for the following purposes:

- Helping to maintain vertical dimension
- Supporting proper speech development
- Improving masticatory function
- Enhancing the child's psychological and social self-confidence (Rodd, H. D., & Barker, C., 2010)

Syndromic or special conditions

They may also be part of a long-term treatment plan for certain disorders affecting oral and dental development (American Academy of Pediatric Dentistry, 2020).

Summary:

Unlike conventional complete dentures, overdentures derive support from retained tooth roots and play an important role in preserving jaw development, particularly in children (Bhat, Shenoy, & Sarpangala, 2013).



Figure 1. Image showing an implant-supported overdenture.

Monolithic CAD/CAM Complete Maxillary Dentures for a Pediatric Patient With Dentinogenesis Imperfecta and Limited Prosthetic Space: A Clinical Report

Children affected by dentinogenesis imperfecta require restorative or prosthetic treatments to reduce both functional problems and psychosocial impact. In cases where primary teeth exhibit significant structural loss and the child's cooperation is limited, removable complete maxillary dentures represent one of the most

suitable treatment options (Millet, Ducret, Khoury, & Virard, 2020).

The planning and fabrication of the complete maxillary dentures were performed using a digital workflow. In this modern monolithic, single-shade design—into which gingiva-colored composite resin was integrated—the approach provided a natural appearance while allowing preservation of the existing restorative space. Such an application requires periodic follow-up of the prosthesis and its replacement when necessary, depending on the child's growth and development as well as the exfoliation timing of primary teeth. Furthermore, long-term management aims to improve the child's compliance with the treatment process to ensure stable rehabilitation (Millet, Ducret, Khoury, & Virard, 2020).

Prosthetic treatment with complete overlay dentures in a pediatric patient with worn dentition: A case report

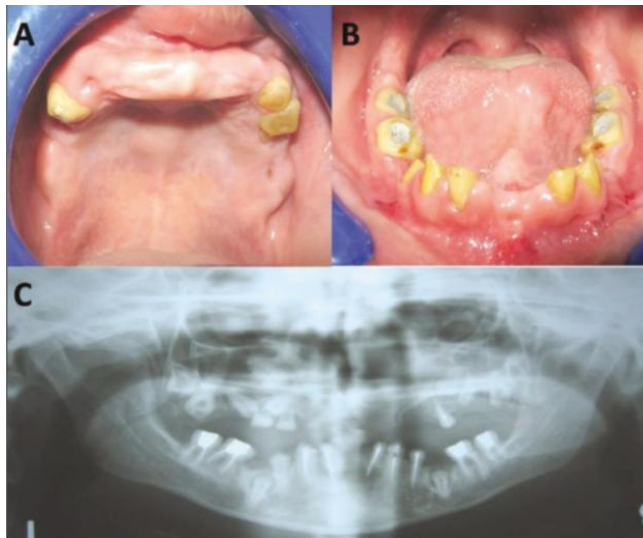


Figure 1. A: Numerous missing teeth and malformed residual teeth are observed in the maxillary arch.

B: In the mandibular arch, partial ankyloglossia, oligodontia, widely spaced teeth, and tooth discoloration are noted.

C: Radiographic appearance of Weyer acrodonal dysostosis. Multiple impacted, unerupted, partially erupted teeth and enlarged pulp chambers are evident. (Kumar, Rastogi, Jain, & Singh, 2012)



Figure 2. Facebow record and intraorally applied overlay overdenture. (Kumar vd., 2012)



Figure 3. The tissue surface of the overlay overdenture shows indentations that adapt to the patient’s remaining teeth in the mouth. (Kumar vd., 2012)



Figure 4. A: Pretreatment appearance
B: Post-treatment appearance. A noticeable increase in lower facial height and a marked improvement in esthetics can be observed. (Kumar vd., 2012)



Figure 5. Post-treatment frontal view. (Kumar vd., 2012)

Dental Implants as a Treatment Option for Prosthetic Rehabilitation in Children Diagnosed With Ectodermal Dysplasia

In ectodermal dysplasia (ED), abnormalities are typically present in at least two ectoderm-derived structures such as the skin, hair, nails, sweat glands, and teeth (Lamartine, 2003). Among these manifestations, dental problems are often the most difficult to manage because they can significantly affect oral health, masticatory function, appearance, and the individual's psychosocial well-being (Nunn et al., 2003).

Considering advances in implant technology and the challenges associated with managing dental anomalies in children with ED, the use of implants must be carefully evaluated as part of the prosthetic treatment options for this special patient group (Raducanu, Papagiannoulis, Oulis, & Yamashiro, 2009).

A review of published cases indicates that all researchers reported favorable maxillofacial growth and development, along with stable implants and prostheses. Reported implant success rates ranged from 88% to 100% over follow-up periods of 3 to 8.1 years (Barve, Padawe, & Takate, 2025). Implant-supported maxillary overdentures and implant-supported fixed prostheses have become key treatment options in individuals with ED, enhancing masticatory efficiency, improving facial aesthetics, and providing long-term stability (Barve, Padawe, & Takate, 2025). Indirect Restorations

Indirect restorations are restorative solutions fabricated outside the mouth—in a laboratory or through CAD/CAM systems—and subsequently cemented onto the tooth. In cases with extensive structural loss, they offer superior mechanical strength and more satisfactory esthetic outcomes compared with direct restorations (Rosenstiel, Land, & Fujimoto, 2016).

Types of Indirect Restorations

Indirect restorations are classified according to their extent and intended use:

Inlay:

Fills a defined cavity on the occlusal surface of the tooth without covering the cusps (Rosenstiel, Land, & Fujimoto, 2016).

Onlay:

Covers one or more cusps, providing a more extensive restoration (Rosenstiel, Land, & Fujimoto, 2016).

Overlay:

Covers the entire occlusal surface (Rosenstiel, Land, & Fujimoto, 2016).

Crown:

A comprehensive restoration that covers all surfaces of the tooth (Rosenstiel, Land, & Fujimoto, 2016).

Laminate veneer:

Thin porcelain veneers applied primarily in the anterior region to enhance esthetics (Rosenstiel, Land, & Fujimoto, 2016).



Figure 1. Diagram illustrating inlay, onlay, and overlay restorations.



Figure 2. Schematic illustration showing inlay, onlay, and overlay restorations.



Figure 3. Image showing inlay and onlay restorations.



Figure 4. Image showing inlay and onlay restorations.

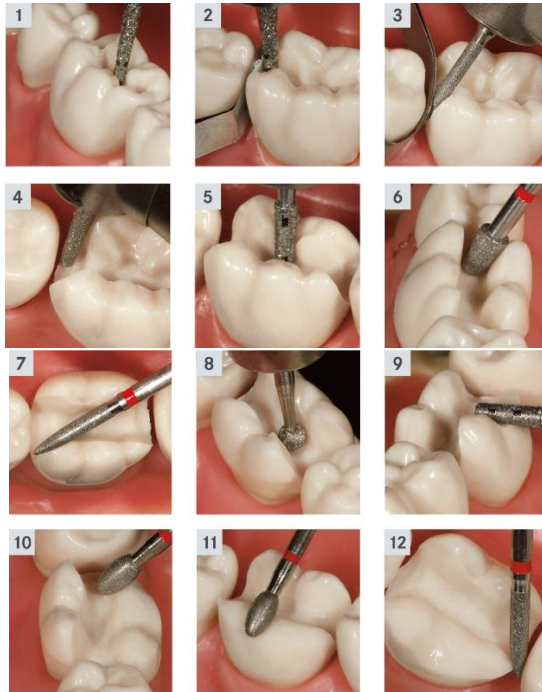


Figure 5. Clinical images showing the stages of inlay cavity preparation.

Materials Used

The primary materials commonly used in indirect restorations include:

- Ceramic types (e.g., feldspathic porcelain, zirconia)
- Composite resin materials
- Metal alloys (such as gold, nickel–chromium)

Ceramics provide superior esthetic performance, whereas metal alloys offer advantages in terms of durability (Anusavice, Shen, & Rawls, 2013).

Advantages

- Provide more precise marginal adaptation
- Offer high mechanical strength
- Produce superior esthetic outcomes
- Polymerization shrinkage is controlled under laboratory conditions
- Result in longer-lasting restorations (Ferracane, 2011)

Disadvantages

- Treatment may take longer and be more costly
- Conventional techniques require at least two separate appointments

- Dependence on a laboratory workflow
- Require the use of a temporary restoration until treatment completion (Rosenstiel, Land, & Fujimoto, 2016)

Indications

- Cases with extensive caries or fractures
- Restorations where direct techniques are insufficient
- Patients with high esthetic expectations
- Structurally weakened teeth following endodontic treatment (Magne & Belser, 2003)

Cementation Process

Commonly used cementation materials for indirect restorations include:

- Resin-based cements (most frequently preferred)
- Glass ionomer cements
- Resin-modified glass ionomer cements

Proper application of adhesive systems is a critical step that directly affects the long-term success of the restoration (Hill & Lott, 2011).

Indirect restorations hold significant value in contemporary dentistry from both functional and esthetic perspectives. With advancements in CAD/CAM technologies, the applicability and success rates of these restorations continue to increase (Miyazaki, Hotta, Kunii, Kuriyama, & Tamaki, 2009).

Clinical Longevity of Direct and Indirect Posterior Resin Composite Restorations: Updated Systematic Review and Meta-analysis

Indirect restorations hold a significant position in contemporary dentistry from both functional and esthetic perspectives. Due to advancements in CAD/CAM technologies, the applicability and success rates of these restorations continue to improve steadily (Worthington et al., 2021).

The annual failure rate of direct resin composite restorations increases in individuals over 65 years of age and in patients using removable prostheses (Laske, Opdam, Bronkhorst, Braspenning, & Huysmans, 2016). Higher failure rates are also reported in molars, in teeth that have undergone endodontic treatment, and in restorations involving four or more surfaces (Demarco et al., 2012).

Although fractures occur with similar frequency in both amalgam and resin composite restorations, resin composites in adult patients have been reported to show a greater tendency toward secondary caries formation (Worthington et al., 2021). The development of secondary caries is largely associated with polymerization shrinkage at the material–tooth interface and the stress that results

from it, whereas fractures can be attributed to the mechanical limitations of the restorative material as well as tooth- and patient-specific factors. This issue becomes particularly evident in large cavity restorations that involve cusps (Ferracane, 2011).

Another significant problem in direct resin composite restorations is the inability to achieve adequate polymerization, as polymerization depth may be compromised by irregular maintenance of curing lights and operator-dependent variables (Cadenaro et al., 2019).

Indirect resin composite restorations can theoretically provide longer-lasting results by mitigating several limitations of the direct technique. These restorations may be fabricated using prefabricated CAD/CAM composite blocks or composite materials prepared in a dental laboratory by a technician. Such materials undergo extended polymerization cycles with light applied from multiple directions, thereby increasing the degree of monomer conversion and markedly enhancing the mechanical strength of the material (Santini, Miletic, Swift, & Bradley, 2012).

Disadvantages of Indirect Restorations

Indirect restorations also present several disadvantages:

Longer treatment duration

- The clinical procedure may take more time.
- In the traditional workflow, an impression must first be taken.
- The impression is then sent to the laboratory.
- At the second appointment, the restoration is cemented.
- These steps require at least two clinical visits.

Higher cost

- Additional laboratory fees are required.
- The materials used—particularly ceramics and zirconia—are costly.
- Clinical application time may be longer.
- For these reasons, indirect restorations are generally more expensive than direct restorations.

Dependence on laboratory procedures

- The workflow depends heavily on laboratory stages.
- Errors may occur during impression taking.
- Problems may arise during the modeling phase.
- Technical issues can occur during fabrication.

All of these factors may adversely affect the fit and overall success of the restoration. Need for temporary restorations

Until the definitive restoration is completed:

- Temporary fillings or provisional crowns are required.
- These temporary restorations may fracture or become dislodged.

More extensive tooth preparation

- In some cases, more tooth structure must be removed compared to direct restorations.

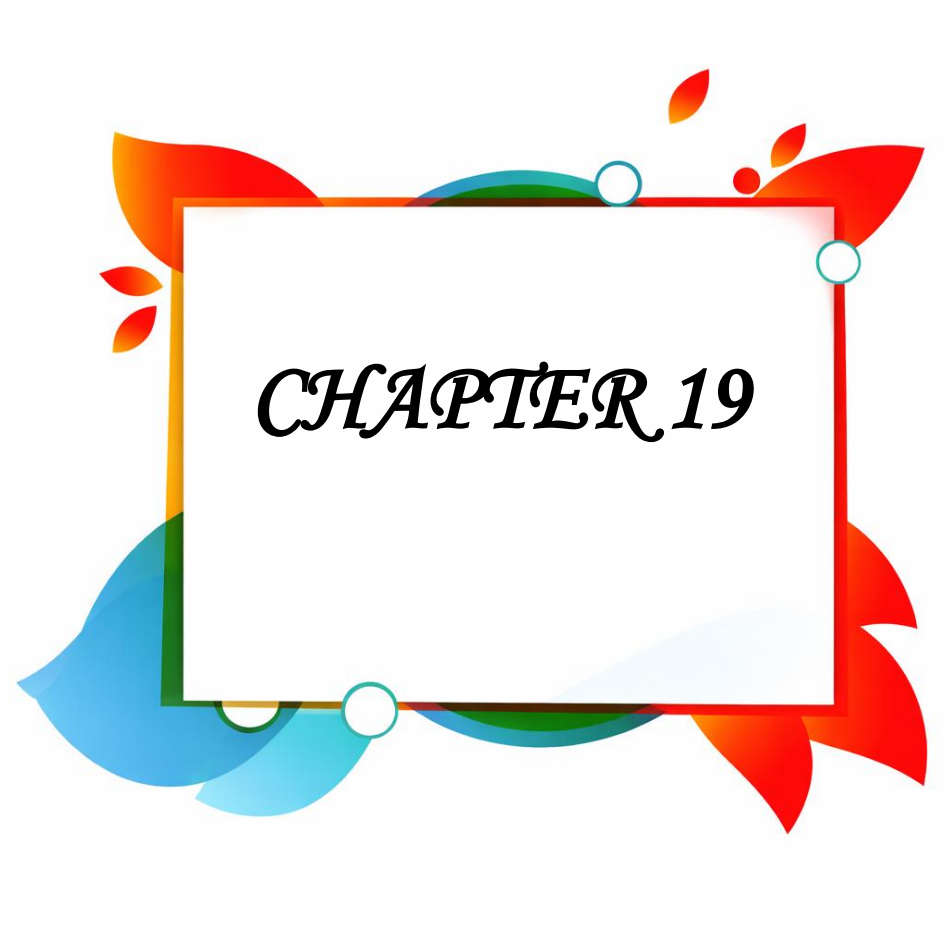
- This may lead to an increased loss of healthy tooth tissue.

Cementation sensitivity

- Adhesive systems require high technique sensitivity.
- Moisture control may be difficult to achieve.
- Errors in the cementation process can result in restoration failure.

Risk of fracture (especially in ceramics)

Although highly esthetic, ceramic restorations may be more prone to fracture in thinner areas. (Rosenstiel, Land, & Fujimoto, 2016; Shillingburg et al., 2012)



Tooth Autotransplantation in Contemporary Dentistry: Biological Principles, Multidisciplinary Challenges, and Future Perspective

Mustafa Üstün¹

INTRODUCTION

Tooth autotransplantation is the surgical relocation of a donor tooth to a different site within the same individual's oral cavity (Tsukiboshi, Tsukiboshi, & Levin, 2023). This procedure serves as a superior biological alternative to conventional dental implants and fixed prostheses, particularly for growing patients who suffer from premature tooth loss due to severe dental trauma or congenital agenesis (Tan et al., 2023). Because a successful autotransplant preserves a vital periodontal ligament, it uniquely promotes continuous dentoalveolar growth, induces functional bone formation, and maintains natural gingival contours (Kafourou et al., 2017). Consequently, it provides a highly predictable, long-term restorative solution.

MATERIALS AND METHOD

1. The Biological Imperative: Periodontal Ligament Viability and Cellular Healing

The biological success of tooth autotransplantation is fundamentally dependent upon the preservation and cellular viability of the periodontal ligament (PDL) and Hertwig's epithelial root sheath (HERS) (Andreasen, Kristerson, & Andreasen, 1988). The PDL harbors stem cells with immense pluripotent and regenerative capacity, which are essential for establishing a new attachment apparatus and driving osteogenic activity at the recipient site (Zhang et al., 2012). Maintaining the vitality of these delicate cells directly prevents severe postoperative complications, most notably external replacement resorption (ankylosis) and infection-related inflammatory root resorption (Machado, do Nascimento et al., 2016; Tan et al., 2023). If the PDL undergoes unfavorable healing or necrosis due to surgical trauma, the long-term prognosis of the transplanted tooth is severely compromised, eventually leading to procedural failure and tooth loss (Kafourou et al., 2017). Furthermore, preserving the integrity of HERS in immature donor teeth is critical, as it stimulates continued

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dentoalveolar growth and physiological root development within the new socket (Wong, Fok, Li, Zhang, & Cheung, 2026).

Because the biological imperative of PDL survival is so high, clinicians must strictly adhere to intraoperative protocols that minimize extra-alveolar time and mechanical trauma. Prolonged extraoral exposure and dehydration rapidly deplete PDL cell viability, which significantly increases the vulnerability of the root to osteoclastic resorptive activity (Hupp, Mesaros, Aukhil, & Trope, 1998). Contemporary guidelines strongly emphasize keeping the extra-alveolar time under 15 minutes to maximize the regenerative potential of the periodontal tissues (Wong et al., 2026). To predictably achieve this, the integration of digital planning, cone-beam computed tomography (CBCT), and three-dimensionally (3D) printed tooth replicas has revolutionized surgical handling (Figure 1). Utilizing these computer-aided rapid prototyping models for initial socket preparation prevents the need for multiple traumatic try-ins of the actual donor tooth, thereby shielding the fragile root surface and its PDL cells from crushing forces (Anssari Moin, Verweij, Waars, van Merkesteyn, & Wismeijer, 2017; Verweij, Jongkees, Anssari Moin, Wismeijer, & van Merkesteyn, 2017).

Figure 3: Individually 3D-printed donor tooth replicas (premolars and molars) that served as a surgical guide for the preparation of the neo-alveolus during 3D autotransplantation (Verwij et al., 2017)



Following atraumatic placement, the cellular healing phase relies heavily on an appropriate postoperative stabilization protocol. Evidence indicates that excessive or highly rigid fixation negatively influences PDL regeneration, thereby increasing the susceptibility to ankylosis and inflammatory root resorption (Gostilo & Jäkobsone, 2025). Instead, a flexible splint—such as a suture splint or a light wire-composite splint—applied for a short duration of 1 to 4 weeks allows for physiological micro-mobility (Bauss, Schilke, Fenske, Engelke, & Kiliaridis, 2002). This slight mobility actively stimulates cellular proliferation, encourages optimal periodontal reattachment, and discourages bony fusion (Tsukiboshi, Tsukiboshi, & Levin, 2023). When these biological principles are meticulously applied to protect the PDL, the autotransplanted tooth can seamlessly integrate, restoring natural proprioception, preserving alveolar bone volume, and providing excellent long-term function (Gault & Warocquier-Clerout, 2002).

2. Esthetic Zone Challenges: Rehabilitating the Anterior Maxilla

Rehabilitating the anterior maxilla poses significant restorative challenges, as tooth loss in this esthetically demanding region—frequently resulting from traumatic dental injuries (TDI) or congenital agenesis—is often accompanied by severe soft tissue collapse and alveolar bone resorption (Grisar et al., 2018). For growing children and adolescents, conventional fixed dental prostheses and osseointegrated implants are generally contraindicated due to their inability to adapt to continuous dentoalveolar growth, which inevitably leads to infraocclusion and functional discrepancies (Akhlef et al., 2024). Consequently, autotransplantation has emerged as a biologically sound and highly predictable alternative. By commonly utilizing developing premolars to substitute missing central or lateral incisors, clinicians can re-establish the periodontium, stimulate natural alveolar bone growth, and seamlessly preserve the surrounding tissue architecture without the developmental risks associated with rigid artificial prosthetics (Stange, Lindsten, & Bjerklin, 2016).

Achieving an optimal "pink and white" esthetic match is the ultimate goal in anterior rehabilitations. Long-term clinical and radiographic evaluations consistently demonstrate that successfully transplanted teeth promote natural papilla regeneration and harmonious gingival contours, yielding highly satisfactory esthetic outcomes (Mendoza-Mendoza, Solano-Reina, Iglesias-Linares, Garcia-Godoy, & Abalos, 2012). To address the morphological differences between a donor premolar and a recipient incisor, conservative restorative interventions such as composite resin build-ups or porcelain veneers are frequently employed to fine-tune the final appearance (Vilhjálmsón, Knudsen, Grung, & Bårdsen, 2011). In highly complex scenarios characterized by extensive three-dimensional bone defects, advanced surgical approaches like dentoalveolar complex autotransplantation have proven highly effective; this technique involves relocating the donor tooth alongside its native vestibular bone, thereby eliminating the need for staged bone augmentation and reducing overall patient morbidity (Khachatrian, Lozada, Handysides, & Zernitckaia, 2026).

Beyond routine premolar transfers, the versatility of autotransplantation extends to the resolution of rare morphological anomalies within the esthetic zone. Clinical reports have documented the successful relocation of supernumerary teeth or the surgical reorientation of inverse impacted dilacerated incisors to restore arch integrity and esthetics (Yavuz, Cekmegeli, & Mentés, 2024). While anterior autotransplants can occasionally suffer from long-term biological complications such as external inflammatory root resorption or ankylosis, the incidence remains relatively low when proper case selection and atraumatic techniques are applied (Gilijamse, Baart, Wolff, Sándor, & Forouzanfar, 2016). Ultimately, the preservation of natural tissue volume and proprioception ensures that patients consistently report high levels of satisfaction regarding both function and esthetics, solidifying the procedure's value in multidisciplinary anterior rehabilitation (Plakwicz, Andreasen, Górská, Burzykowski, & Czochrowska, 2021).

3. Navigating Complex and Rare Dental Anomalies

Beyond standard tooth replacements for trauma or agenesis, autotransplantation proves exceptionally valuable for managing rare morphological anomalies and ectopic eruptions. Clinicians have successfully utilized this procedure to resolve severe developmental malformations by repurposing supernumerary teeth or supplemental premolars to replace fused incisors or teeth with abnormal dimensions (Cho & Lee, 2007). Moreover, highly complex impactions, such as inverse impacted dilacerated incisors, can be surgically extracted, correctly reoriented, and autotransplanted into their proper anatomical position. Long-term follow-ups of such uniquely challenging cases demonstrate excellent functional survival and preservation of alveolar bone, confirming that severely deformed teeth can still serve as viable donors when handled atraumatically (Yavuz, Cekmegeli, & Menten, 2024).

The versatility of tooth autotransplantation further extends to rehabilitating severe dentoalveolar defects and craniofacial malformations. For patients with cleft lip and palate, transplanted teeth can be successfully relocated into bone-grafted alveolar clefts, facilitating natural bone induction, periodontal ligament stimulation, and arch continuity where conventional prosthetics often fail (Naros, Schulz, Finke, Reinert, & Krimmel, 2024). Additionally, autotransplantation has been creatively applied to immediately close complex anatomical perforations, such as oro-antral communications, utilizing mature third molars as biological plugs to seal the defect while restoring the occlusal table (Nagori, Jose, Bhutia, & Roychoudhury, 2015). In instances of severe maxillary atrophy, autotransplantation can even be performed simultaneously with sinus floor elevation and allogenic bone grafting to successfully restore function in heavily compromised ectopic sites (Pang, Choi, Kim, & Park, 2011).

Finally, while the procedure is typically utilized for single-tooth defects, advanced protocols now permit comprehensive rehabilitation through multiple-tooth autotransplantation. Recent clinical studies tracking adult patients who required the simultaneous or sequential replacement of three or more teeth demonstrated long-term survival rates exceeding 92% and success rates of 84.6% over observation periods spanning up to 8 years (Tiigimäe-Saar, 2023). When combined with modern computer-aided design (CAD) and three-dimensional surgical guides, multiple dental autotransplantations can be meticulously planned prior to surgery. This digital approach ensures optimal spacing, proper occlusion, and precise root positioning across the arch, confirming that autotransplantation is a robust, holistic treatment modality capable of addressing extensive and complex oral rehabilitations (Criado Villalón, Fernández González, Medina del Valle, & Sáez-Torres, 2024).

4. The Intersection of Autotransplantation and Orthodontic Management

The intersection of dental autotransplantation and orthodontic therapy provides a highly symbiotic approach to managing complex malocclusions compounded by missing teeth. In patients presenting with traumatic tooth loss or congenital agenesis, orthodontic space management alone may compromise the facial profile or arch integrity (Schatz & Joho, 1994). When a patient simultaneously requires the extraction of healthy premolars or third molars to resolve crowding, these teeth can be strategically repurposed as donor grafts to fill the edentulous regions (Kvint, Lindsten, Magnusson, Nilsson, & Bjerklin, 2010). Clinical evaluations confirm that once the autotransplanted tooth biologically integrates, it responds to orthodontic biomechanics identically to a natural tooth, allowing clinicians to correct midline deviations, optimize interproximal contacts, and establish functional occlusion (Piroozmand, Hessari, Shirazi, & Khazaiecor-responding, 2018). Furthermore, long-term observations of orthodontic patients who received autotransplanted teeth with complete root formation demonstrate excellent survival and aesthetic harmony, maintaining intact alveolar ridges and negating the need for artificial prostheses (Watanabe et al., 2010).

The timing and magnitude of orthodontic forces applied to an autotransplanted tooth are critical determinants of its long-term pulpal and periodontal health. Contemporary guidelines generally recommend initiating orthodontic tooth movement after a brief stabilization period, ensuring that initial periodontal ligament healing and, if applicable, pulpal revascularization are successfully established (Lacerda-Santos et al., 2020). Studies evaluating the specific effects of orthodontic derotation and extrusion on immature donor teeth have shown that controlled forces do not significantly increase the risk of pulpal necrosis or severe root resorption, provided the biological limits of the regenerating tissues are respected (Bauss, Schwestka-Polly, & Kiliaridis, 2004). However, excessive or premature force application may disturb the delicate Hertwig's epithelial root sheath or strangulate the apical neurovascular bundle, potentially arresting root development in immature transplants (Lagerström & Kristerson, 1986). When managed with meticulous interdisciplinary coordination, guided orthodontic movement of autotransplants can effectively regenerate alveolar bone width and height, maximizing the restorative potential in the compromised recipient site (Abella Sans, Ribas, Doria, Roig, & Durán-Sindreu, 2021).

5. Adjunctive Biological Materials and Tissue Engineering

To optimize osseointegration and soft-tissue healing in recipient sites with severe anatomical deficiencies, clinicians are increasingly combining tooth autotransplantation with adjunctive biological materials and guided bone regeneration (GBR). When confronting alveolar atrophy or pneumatized sinuses, the simultaneous application of autogenous bone grafts, allogenic grafts, or sinus floor elevation techniques can successfully reconstruct the compromised three-dimensional architecture (Bauss, Engelke, Fenske, Schilke, & Schwestka-Polly,

2004; Pang, Choi, Kim, & Park, 2011). Furthermore, the application of guided tissue regeneration (GTR) membranes and enamel matrix derivatives, such as Emdogain, onto root surfaces has been proposed to enhance the proliferation of vital periodontal ligament (PDL) cells and mitigate the risk of replacement resorption (Pedrinaci et al., 2024). At the frontier of tissue engineering, experimental therapies utilizing compact bone-derived cell spheroids and targeted PDL cell transplantation demonstrate remarkable potential to promote neo-bone formation and establish a functional biomimetic attachment apparatus around the transplanted root (Matsumura et al., 2022).

In addition to structural bone grafts, the integration of autologous platelet concentrates has revolutionized the biological stimulation of healing sockets. Biological scaffolds such as leukocyte- and platelet-rich fibrin (L-PRF) or concentrated growth factors release a steady cascade of osteogenic and angiogenic proteins that accelerate the maturation of both soft and hard tissues, even in areas previously affected by periapical inflammation (Keranmu, Ainiwaer, Nuermuhanmode, & Ling, 2021). Advancements in centrifugation protocols have also introduced injectable and advanced platelet-rich fibrin (i-PRF and A-PRF), which can be combined directly with bone particulate to create a highly bioactive, moldable "sticky bone" matrix (Salinas Molina, Cevallos Calero, & Rodríguez Reyes, 2026). Clinical evidence confirms that enriching the surgically created socket with these autologous fibrin networks dramatically improves early stabilization, minimizes post-surgical morbidity, and offers a promising adjunct for achieving predictable periodontal regeneration (SamavatiJame, Alrmali, Galindo-Fernández, Stuhr, & Wang, 2025).

Finally, the long-term biological success of mature autotransplants frequently depends on the strategic use of advanced endodontic materials for extraoral apical sealing. Calcium silicate-based bioceramic cements, such as Biodentine, are highly favored for root-end fillings and retrograde seals due to their superior biocompatibility, osteoconductivity, and ability to stimulate reparative hard tissue formation (Malkondu, Kazandağ, & Kazazoğlu, 2014). The excellent marginal adaptation and washout resistance of these materials ensure a hermetic barrier against bacterial microfiltration, a critical factor when the tooth is exposed to the humid environment of the neo-alveolus (Falkowska et al., 2023). However, while the combination of bioceramics and regenerative techniques like i-PRF is theoretically ideal, clinicians must maintain rigorous infection control, as the presence of latent bacteria or inadequate biomechanical preparation can still lead to persistent inflammation and unfavorable procedural outcomes (Lazo Espinoza, Rodríguez Reyes, & Lugo Pinto, 2026).

6. Comparative Efficacy: Autotransplantation vs. Osseointegrated Implants

When evaluating restorative options for missing or irretrievably compromised teeth, tooth autotransplantation (AT) demonstrates distinct biological advantages over traditional osseointegrated dental implants, particularly in pediatric and

adolescent populations (García-Miralles et al., 2025). Dental implants achieve absolute rigid fixation within the bone—behaving essentially as ankylosed elements—which prevents them from participating in the continuous dentoalveolar growth of a developing jaw (Akhlef et al., 2025). Consequently, placing implants in growing patients frequently leads to severe infraocclusion, functional discrepancies, and significant aesthetic deformities over time (Tsukiboshi, Tsukiboshi, & Levin, 2023). In contrast, successfully autotransplanted teeth preserve a vital periodontal ligament that induces physiological bone remodeling, allows the tooth to erupt in harmony with the adjacent dentition, and maintains natural proprioception (Figure 2 and 3) (Dokova et al., 2024).

Figure 4: **A.** Initial panoramic radiograph showing open apices on the mandibular second premolars. **B.** Pretreatment intraoral photograph showing a lack of vertical space for the transplants. **C.** Pretreatment intraoral photograph showing a lack of horizontal space for the transplants. (Dokova et al., 2024)

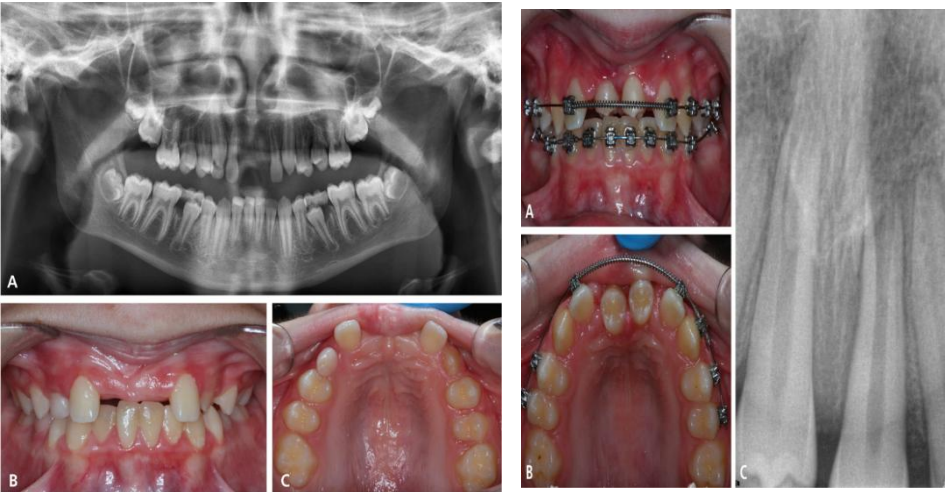


Figure 5: Intraoral photographs 3 weeks postsurgery. **A.** Open coil springs were used to increase horizontal space for transplanted teeth. **B.** The curve of Spee was leveled with lower appliances to create vertical space. **C.** Orthodontic force was applied to the donor premolars 4 weeks before surgery to stimulate the periodontal ligament and aid in atraumatic extraction of the transplant teeth. There are open apices on the transplanted teeth. (Dokova et al., 2024)

From an anatomical and esthetic perspective, autotransplantation often yields superior integration in visually demanding areas compared to implant-supported prostheses. While single-tooth implants in the anterior maxilla frequently encounter complications related to insufficient papilla fill, unesthetic gingival margins, and mismatching crown colors, autotransplanted teeth inherently maintain natural soft tissue trophism and harmonious gingival contours (Boschini, Melillo, & Berton, 2020). Furthermore, in scenarios presenting with severe alveolar bone resorption, the placement of a dental implant typically

mandates invasive, staged bone augmentation procedures. Conversely, advanced AT variations—such as dentoalveolar complex transplantation—can simultaneously transfer a donor tooth alongside its native vestibular bone, biologically reconstructing the deficient site and providing a highly predictable alternative to complex implant grafts (Khachatryan, Lozada, Handysides, & Zernitckaia, 2026).

Finally, the long-term strategic and economic efficacies of AT make it a highly competitive alternative to implantology. From a financial standpoint, autotransplantation is generally more cost-effective than the cumulative expenses associated with implant placement, abutments, and prosthetic crowns (Tan et al., 2023). Moreover, even if an autotransplanted tooth eventually experiences late-stage failure due to replacement resorption or ankylosis, it serves as an excellent biological space maintainer during its functional lifespan. This process preserves or even enhances the three-dimensional volume of the alveolar ridge, effectively preparing the site for straightforward, future implant placement in adulthood without the need for adjunctive regenerative surgeries (Tiigimäe-Saar, 2023).

7. Standardization of Care: Clinical Guidelines and Methodological Quality

Despite the high predictability of tooth autotransplantation (AT), its widespread adoption among general dentists, pediatric dentists, and orthodontists relies heavily on the establishment of evidence-based clinical guidelines (Çelik & Camcı, 2025; Dokova et al., 2024). Major professional organizations, including the European Society of Endodontology (ESE) and the International Association of Dental Traumatology (IADT), have developed comprehensive position statements and clinical protocols to standardize surgical procedures and expected outcomes (Fouad et al., 2020). However, significant variability remains in the literature regarding the criteria used to define clinical "success" versus "survival." To address this inconsistency, researchers increasingly advocate for the implementation of core outcome sets (COSET) and validated patient-reported outcome measures (PROMs), such as the Oral Health Impact Profile (OHIP-14), to ensure that both biological healing and patient-centered quality of life are uniformly evaluated across clinical practices (Wong, Fok, Li, Zhang, & Cheung, 2026).

A critical appraisal of the current literature reveals that the methodological quality of studies investigating AT is frequently characterized by a high risk of bias. Umbrella reviews and meta-analyses consistently report that the evidence base is dominated by retrospective cohort studies and case series, with a conspicuous absence of well-designed randomized controlled trials (RCTs) (Tan et al., 2023). Methodological assessments utilizing strict appraisal tools—such as the Methodological Index for Non-Randomized Studies (MINORS), the Risk Of Bias In Non-randomized Studies of Interventions (ROBINS-I), and the Joanna Briggs Institute criteria—frequently downgrade the certainty of evidence due to small sample sizes, lack of control groups, and non-standardized follow-up intervals (Machado, do Nascimento, Ferreira, Mattos, & Vilella, 2016).

Furthermore, evaluations of the systematic reviews themselves using the AMSTAR 2 and ROBIS instruments indicate that many reviews suffer from critically low quality, often failing to account for publication bias or to adequately explain the exclusion of potentially relevant primary studies (Jaber et al., 2024).

To elevate the strength of clinical recommendations, future research must adhere strictly to established reporting guidelines and robust methodological frameworks. Adherence to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement and the prospective registration of study protocols in databases like PROSPERO are essential steps for improving transparency and reproducibility (García-Miralles et al., 2025). Similarly, case reports and *in vitro* studies should comply with the CARE and CRIS guidelines, respectively, to enhance the reliability of their findings (Verweij, Jongkees, Anssari Moin, Wismeijer, & van Merkesteyn, 2017). Ultimately, the application of the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) approach highlights the urgent need for prospective, multicenter RCTs with universally accepted follow-up methodologies, which will refine surgical protocols and firmly establish AT as an evidence-based standard of care worldwide (Bouchghel, Khamlich, Bourzgui, & El Quars, 2022).

CONCLUSION

Tooth autotransplantation stands as a highly predictable and biologically superior alternative to conventional prostheses and osseointegrated implants, particularly for growing patients facing premature tooth loss. Its foundational success is driven by the careful preservation of a vital periodontal ligament, which uniquely enables the transplanted tooth to adapt to continuous dentoalveolar growth, induce natural bone regeneration, and maintain harmonious gingival aesthetics. Beyond routine single-tooth replacements, the procedure offers remarkable versatility in multidisciplinary dentistry. It successfully addresses complex developmental anomalies, rehabilitates severe traumatic injuries in the esthetic zone, and seamlessly integrates with orthodontic treatments to restore both arch integrity and optimal occlusal function.

Despite its proven clinical efficacy, excellent long-term survival rates, and economic advantages, the widespread global adoption of autotransplantation requires continued evolution and standardization. The recent integration of digital planning, 3D-printed donor replicas, and adjunctive biological scaffolds has already revolutionized surgical safety by minimizing extraoral time and protecting delicate root tissues. Moving forward, the dental community must focus on overcoming current methodological limitations in the literature. By establishing universally accepted clinical guidelines and prioritizing high-quality, prospective randomized controlled trials, practitioners can firmly elevate tooth autotransplantation from a specialized, underutilized technique to a mainstream, evidence-based standard of care.

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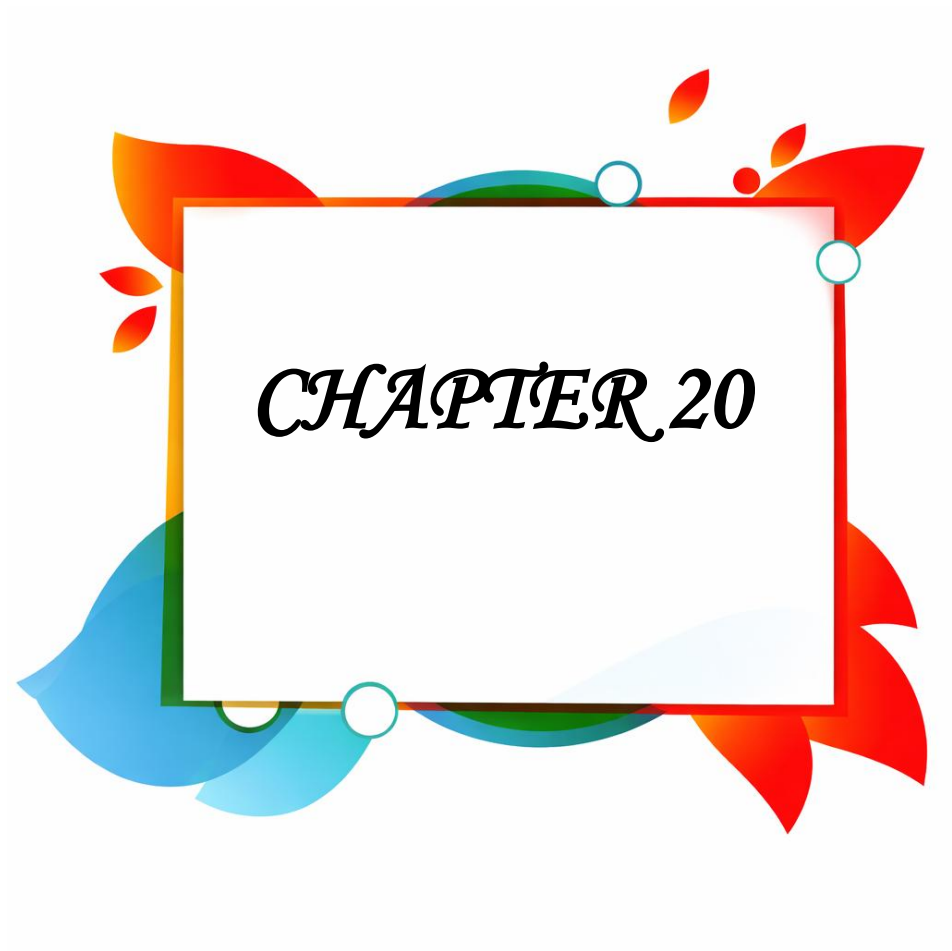
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Histopathological Spectrum of Celiac Disease

Seda Tas Ayçiçek¹

Celiac disease (CD) is a chronic immune-mediated enteropathy that develops in genetically predisposed individuals following gluten ingestion and is characterized by distinctive histopathological changes in the small intestinal mucosa as well as specific serological findings (Fasano & Catassi, 2012).

In recent years, knowledge regarding the diagnosis, pathogenesis, and natural course of CD has expanded significantly, accompanied by notable changes in its epidemiological features. In particular, the widespread use of highly sensitive and specific serological screening tests has enabled more effective identification of at-risk populations and has led to a marked increase in the number of diagnosed cases, including in older age groups beyond childhood. Although the global prevalence of celiac disease is currently estimated to be approximately 1%, the true prevalence is thought to be higher due to a substantial number of undiagnosed cases (Caio et al., 2019).

1. EPIDEMIOLOGY

Celiac disease is among the most common immune-mediated disorders worldwide, with an estimated prevalence of approximately 1% in the general population. However, its distribution varies across different geographic regions. The prevalence is significantly lower in populations where HLA-DQ2 and HLA-DQ8 genotypes are less frequent and dietary gluten consumption is relatively low.

Epidemiological studies have shown that celiac disease occurs more frequently in specific at-risk groups. Individuals with a first-degree relative affected by celiac disease, as well as patients with Down syndrome, type 1 diabetes mellitus, and selective IgA deficiency, have a significantly increased risk compared with the general population. Therefore, closer clinical and serological evaluation is recommended in these groups (Caio et al., 2019; Fasano & Catassi, 2012).

2. PATHOGENESIS

Genetic predisposition, environmental triggers, and immunological mechanisms all play a central role in the development of celiac disease. The main environmental trigger is gluten, a protein complex composed of prolamins found in wheat, barley, and rye. Due to its high proline and glutamine content, gluten peptides cannot be fully degraded by gastrointestinal enzymes, thereby initiating

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an abnormal immune response in genetically susceptible individuals (Lindfors et al., 2019; Shan et al., 2002). These peptides reach the lamina propria of the small intestinal mucosa, where they undergo deamidation by tissue transglutaminase (TG2). The resulting negatively charged peptides exhibit increased affinity for HLA-DQ2 and HLA-DQ8 molecules, leading to activation of CD4+ T-cell responses via antigen-presenting cells. Activated T cells trigger the release of proinflammatory cytokines such as interferon- γ , interleukin-21, and tumor necrosis factor- α , initiating mucosal inflammation. This process underlies the characteristic histopathological changes in the small intestine, including villous atrophy, crypt hyperplasia, and intraepithelial lymphocytosis. In parallel, B-cell activation leads to the production of autoantibodies against tissue transglutaminase. Anti-TG2 antibodies, in particular, can be detected in both serum and mucosal tissues (Schumann et al., 2017).

Recent studies have also suggested that anti-TG2 antibodies are not solely produced locally in the gut, but may also reflect a systemic immune response. In addition, autoantibodies targeting different members of the transglutaminase family have been associated with extraintestinal manifestations of celiac disease. For example, TG3 antibodies are linked to dermatitis herpetiformis, while TG6 antibodies are associated with gluten-related neurological involvement (Schumann et al., 2017).

3. CLINICAL FEATURES

Celiac disease is recognized as an immune-mediated enteropathy presenting with a broad clinical spectrum in the adult population. The clinical picture demonstrates heterogeneous features depending on the nature and severity of symptoms as well as the presence of systemic involvement. In 2011, the Oslo classification defined the classical, atypical, subclinical, potential, and refractory forms of celiac disease (Ludvigsson et al., 2013). This clinical subclassification provides an important framework, particularly in adult patients, for guiding the diagnostic process, determining the need for biopsy, and accurately interpreting histopathological findings.

- **Classical (Typical) Celiac Disease**

Classical celiac disease is defined as the clinical form in which malabsorption findings are predominant. Patients with this phenotype commonly present with chronic diarrhea, steatorrhea, weight loss, abdominal distension, and various vitamin and mineral deficiencies (Bai et al., 2013; Fasano & Catassi, 2012; Lebwohl et al., 2018). Serological testing typically shows high positivity for anti-tissue transglutaminase IgA (anti-TG2 IgA) and anti-endomysial antibodies (EMA). Histopathological evaluation reveals marked intraepithelial lymphocytosis, prominent crypt hyperplasia, and advanced villous atrophy; in a significant proportion of cases, Marsh–Oberhuber stage 3B or 3C lesions are observed (Rubio-Tapia et al., 2013). In classical celiac disease, clinical manifestations, serological findings, and histopathological features are generally concordant, forming a consistent diagnostic profile (Lebwohl et al., 2018).

- **Atypical (Non-classical) Celiac Disease**

This is one of the most frequently encountered clinical presentations, particularly in the adult population. In this form, gastrointestinal symptoms may be mild or completely absent. Patients often present to healthcare facilities with extraintestinal manifestations such as iron-deficiency anemia, osteopenia or osteoporosis, recurrent aphthous stomatitis, chronic fatigue, peripheral neuropathy, infertility, and various dermatological findings. Dermatitis herpetiformis, considered a cutaneous manifestation of celiac disease, is also an important condition associated with the non-classical form (Fasano & Catassi, 2012; Lebwohl et al., 2018). Therefore, celiac disease should be included in the differential diagnosis in the presence of unexplained extraintestinal findings, and appropriate serological and histopathological evaluation should be performed.

- **Silent (Subclinical) Celiac Disease**

Silent (subclinical) celiac disease is a clinical form in which there are no apparent clinical symptoms, but serological tests reveal celiac disease-associated antibody positivity and small intestinal biopsy demonstrates characteristic histopathological changes (Fasano & Catassi, 2012). These cases are most commonly diagnosed not based on symptoms, but during screening of high-risk groups or investigations performed for other indications. Individuals with a first-degree relative affected by celiac disease, patients with type 1 diabetes mellitus or autoimmune thyroid disease, and those evaluated for osteoporosis represent groups in which silent celiac disease is more frequently detected. In addition, duodenal biopsies obtained during upper gastrointestinal endoscopy performed for various indications also contribute to the identification of these patients. Histopathological examination reveals enteropathic changes consistent with celiac disease, although the severity of findings may vary between patients.

- **Potential Celiac Disease**

Potential celiac disease refers to a clinical condition in which serological markers specific for celiac disease are positive, whereas small intestinal biopsy does not demonstrate villous atrophy. In these patients, histopathological examination may be entirely normal or may show only mild mucosal alterations (Caio et al., 2019). Diagnostic criteria include positivity for anti-tissue transglutaminase (anti-TG2) and/or anti-endomysial antibodies (EMA), together with the presence of genetic predisposition such as HLA-DQ2 or HLA-DQ8. The absence of characteristic villous atrophy represents the key distinguishing feature of this form. A proportion of individuals with potential celiac disease may progress over time to develop overt enteropathy with typical histopathological changes. Therefore, clinical and serological findings should always be interpreted in conjunction with histopathological evaluation, and close follow-up is recommended when appropriate. In routine pathology practice, this form highlights that normal or near-normal biopsy findings do not entirely exclude celiac disease.

- **Refractory Celiac Disease**

Refractory celiac disease is defined as the persistence of clinical symptoms and villous atrophy in the small intestinal mucosa despite adherence to a strict gluten-free diet for at least 12 months (Malamut & Cellier, 2019). This condition reflects persistent enteropathy and is considered one of the most severe forms of celiac disease. Refractory celiac disease is associated with an increased risk of serious complications, including ulcerative jejunoileitis, collagenous sprue, and particularly enteropathy-associated T-cell lymphoma. Therefore, patients who continue to exhibit symptoms and histopathological abnormalities despite dietary compliance require careful evaluation and close clinical follow-up (Malamut & Cellier, 2019).

4. HISTOPATHOLOGY

Celiac disease is an immune-mediated enteropathy of the small intestinal mucosa triggered by gluten, and it is characterized by distinctive histopathological changes. For accurate interpretation of these alterations, knowledge of the normal histological features of the small intestinal mucosa is essential. In normal duodenal mucosa, slender and elongated villi together with regularly arranged crypts are observed. Histological evaluation requires the presence of at least 3–4 consecutive preserved villi and a normal villus-to-crypt ratio for a biopsy to be considered non-pathological (Serra & Jani, 2006). Under physiological conditions, the villus/crypt ratio typically ranges between 3:1 and 5:1, reflecting the balance of mucosal renewal. While villi are shorter in the duodenum, they progressively become longer toward the jejunum and ileum. In normal small intestinal mucosa, intraepithelial lymphocyte (IEL) density decreases from the base of the villus toward the tip, a pattern referred to as “decrescendo” (Goldstein, 2004). In contrast, the IEL distribution pattern observed in celiac disease is described as “crescendo” (Özakıncı et al., 2020).

In celiac disease, the morphological spectrum of mucosal injury varies depending on the duration of gluten exposure, the intensity of the immune response, genetic susceptibility, and accompanying environmental factors. The histopathological diagnostic accuracy of celiac enteropathy relies on a systematic evaluation of key mucosal changes, which are assessed based on three principal parameters (Bao & Bhagat, 2012):

1. Increased intraepithelial lymphocytes (IELs)
2. Crypt hyperplasia
3. Villous architectural distortion

Classically, the small intestinal mucosa demonstrates shortening and blunting of villi, accompanied by marked crypt hyperplasia. Although mucosal thickness is generally preserved, a significant reduction in the villus/crypt ratio occurs due to villous loss. These three fundamental histopathological changes constitute the basis of the Marsh classification and reflect the morphological counterpart of gluten-sensitive enteropathy. Histopathological alterations may often show a patchy distribution. Therefore, adequate sampling is recommended to improve

diagnostic accuracy (Brown et al., 2021). Current approaches suggest that at least 4–6 duodenal biopsy specimens should be evaluated. The localization of lesions is also diagnostically important. Isolated intraepithelial lymphocytosis without villous atrophy may be observed in the duodenal bulb, whereas villous atrophy may be more pronounced in the distal duodenum. The proximal jejunum is generally considered the site of more severe mucosal involvement. Volta et al. (2020) reported that bulb-only celiac disease accounts for approximately 10–20% of cases (Volta et al., 2020). Therefore, current guidelines recommend inclusion of duodenal bulb biopsies in routine sampling (Brown et al., 2021). Otherwise, biopsies obtained solely from the distal duodenum may lead to missed diagnoses and an increased risk of false-negative results.

Histopathological Classification

In celiac disease, mucosal injury induced by gluten exposure does not present as a uniform pattern but rather as a spectrum of histopathological changes with varying severity. In order to systematically classify these alterations, Marsh proposed a model in which mucosal lesions are evaluated in sequential stages (Marsh, 1992).

One of the earliest histopathological findings in the small intestinal mucosa is an increase in intraepithelial lymphocytes (IELs). This change may occur without significant distortion of villous architecture and is defined as Marsh type 1 (infiltrative lesion). In this stage, the crypt structure and villus/crypt ratio are generally preserved. Clinically, this finding may be associated with early-stage celiac disease, minimal gluten exposure, or latent forms of the disease (Marsh, 1992).

As mucosal injury progresses, proliferative changes may develop in the crypt architecture. This stage, characterized by largely preserved villous structures accompanied by crypt hyperplasia, is defined as Marsh type 2 (hyperplastic lesion). It reflects increased mucosal immune activation and impaired epithelial regenerative dynamics (Marsh, 1992).

In more advanced stages of the disease, villous architecture becomes markedly distorted. Along with intraepithelial lymphocytosis and crypt hyperplasia, villous shortening and flattening develop. This pattern is defined as Marsh type 3 (destructive lesion) and represents the most typical histopathological appearance of celiac disease. Marsh type 3 lesions may be further subclassified according to the severity of villous atrophy into mild, subtotal, and total atrophy (Marsh, 1992).

Marsh type 4 is a rare entity characterized by crypt hypoplasia and severe mucosal atrophy. In current literature, this form is more limited as a distinct clinical category and is generally considered to represent advanced atrophic disease (Marsh, 1992).

In histopathological evaluation, different cutoff values have been proposed for intraepithelial lymphocyte (IEL) counts, and in duodenal mucosa an upper limit of approximately 25 IELs per 100 enterocytes is generally accepted as normal. In

subsequent modifications of the Marsh classification (Oberhuber revision), type 3 lesions were subdivided according to the degree of villous damage.

As a result of these modifications, the Modified Marsh classification reached its final form (Bai et al., 2013; Oberhuber et al., 1999).

Table 1. Modified Marsh (Oberhuber) Classification of Celiac Disease(Oberhuber et al., 1999)

Modified Marsh Classification	
Marsh Type	Histopathological Features
Type 0	Normal mucosa with preserved villous architecture, normal crypts, and normal intraepithelial lymphocyte (IEL) count.
Type 1	Preserved villous architecture with normal villus/crypt ratio and increased intraepithelial lymphocytes (IELs).
Type 2	Increased intraepithelial lymphocytes with crypt hyperplasia; villous architecture is preserved.
Type 3A	Mild villous atrophy with crypt hyperplasia and increased IELs.
Type 3B	Subtotal villous atrophy with crypt hyperplasia and increased IELs.
Type 3C	Total villous atrophy with crypt hyperplasia and increased IELs.

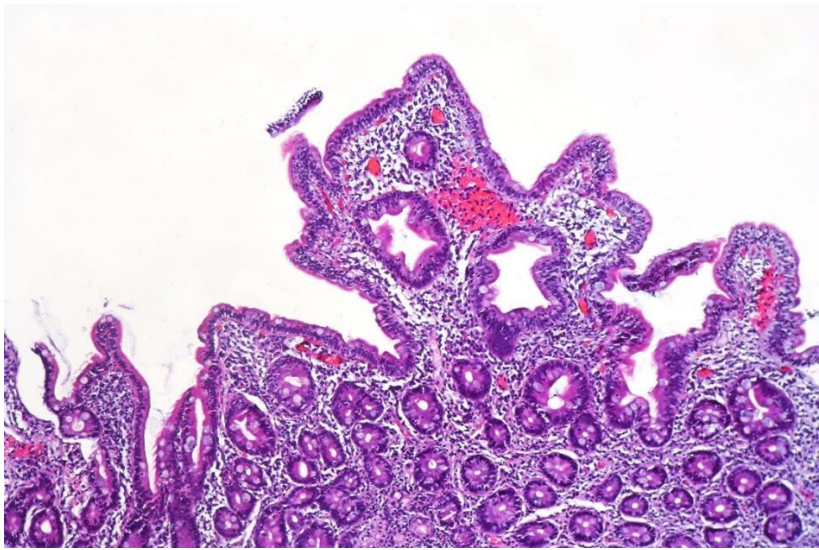


Figure 1. Gluten-sensitive enteropathy (Marsh 3B): Duodenal mucosa demonstrating increased intraepithelial lymphocytes, crypt hyperplasia, and moderate villous atrophy on hematoxylin and eosin (H&E) staining. Source: Wikimedia Commons, CC BY-SA 4.0. Available at: [https://commons.wikimedia.org/wiki/Category:Histopathology_of_coeliac_disease_\(duodenum\)](https://commons.wikimedia.org/wiki/Category:Histopathology_of_coeliac_disease_(duodenum))

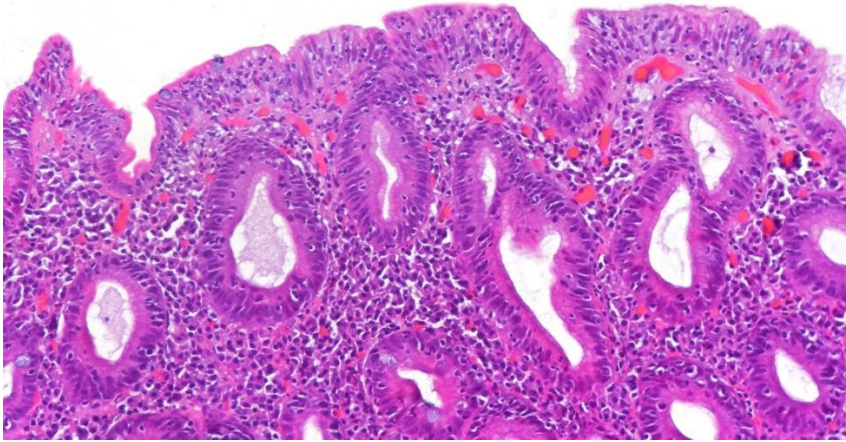


Figure 2. Gluten-sensitive enteropathy (Marsh 3C): Duodenal biopsy showing marked intraepithelial lymphocytosis, crypt hyperplasia, and severe villous atrophy on hematoxylin and eosin (H&E) staining. Source: Wikimedia Commons, CC BY-SA 4.0. Available at: [https://commons.wikimedia.org/wiki/Category:Histopathology_of_coeliac_disease_\(duodenum\)](https://commons.wikimedia.org/wiki/Category:Histopathology_of_coeliac_disease_(duodenum))

5. TREATMENT

Currently, the only evidence-based effective treatment for celiac disease is strict lifelong adherence to a gluten-free diet. This approach allows mucosal healing and effective control of clinical symptoms. However, various pharmacological agents and alternative therapeutic strategies have been investigated for many years, but no effective option has yet entered routine clinical practice. Therefore, desensitization-based approaches aimed at inducing tolerance to gluten remain in the experimental stage (Tack et al., 2013).

Alterations in the gut microbiota of patients with celiac disease have also attracted increasing attention in recent years, and modulation of the microbiome for therapeutic purposes is considered a potential future treatment strategy (Doyle et al., 2025).

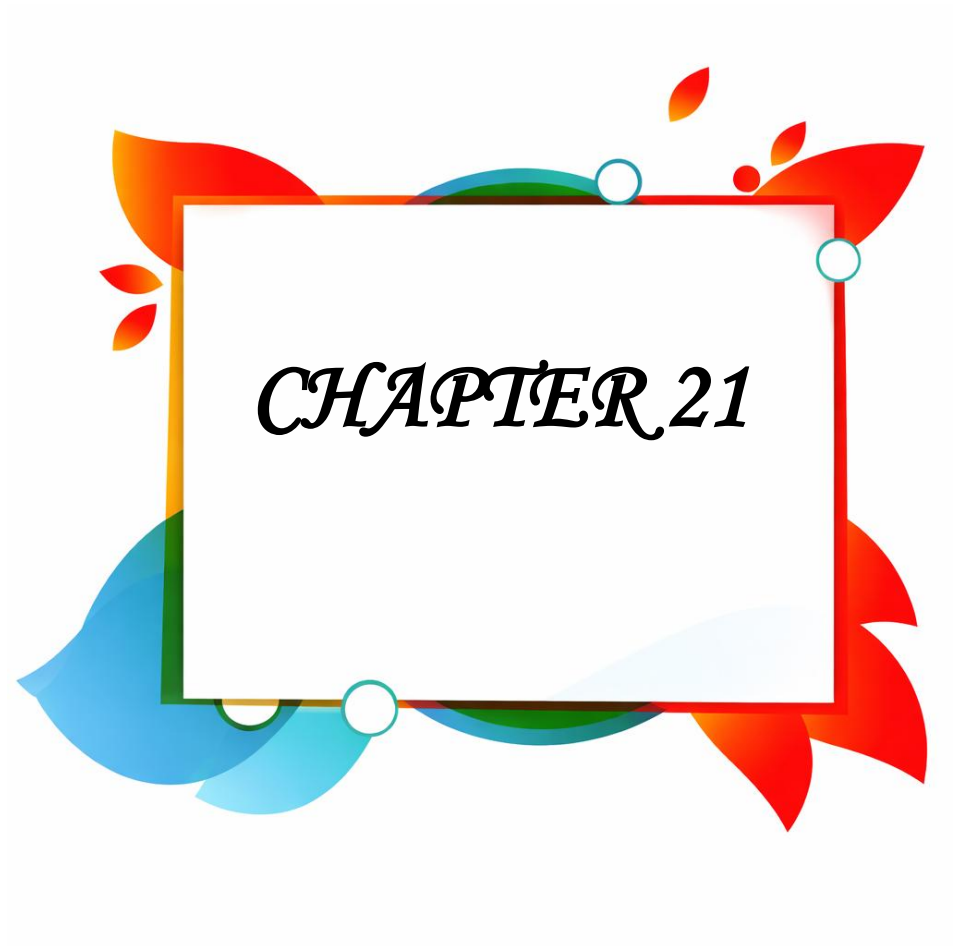
Among pharmacological approaches, latiglutenase is an enzymatic agent targeting gluten digestion. Phase II clinical trials have suggested that it may reduce symptoms following gluten exposure; however, it has no established role in current clinical practice (Syage et al., 2017).

Similarly, prolyl endopeptidases and various gluten-specific endoproteases represent enzymatic treatment options designed to degrade gluten proteins and reduce their immunogenic load. Nevertheless, the clinical efficacy of these agents has not yet been sufficiently demonstrated, and they are not included in current standard treatment algorithms (Tack et al., 2013).

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Biomechanics of The Circulatory System

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INTRODUCTION

The biomechanics of the circulatory system is an interdisciplinary field that encompasses the investigation of fluid motion occurring throughout the heart, blood vessels, and lymphatic system, as well as the mechanical forces acting on these structures. The cardiovascular system can be considered a complex mechanical structure in which a biological pump and an elastic tubular system operate in an integrated manner. In this system, blood flow is determined not only by pressure gradients but also by the viscoelastic properties of vessel walls, the contractile characteristics of the myocardial muscle, and the rheological behavior of the fluid (Hoskins et al., 2017; Karakoç & Naderi, 2020).

Understanding the biomechanical properties of the circulatory system is critically important for explaining the mechanisms underlying pathological conditions such as hypertension, heart failure, atherosclerosis, and lymphedema.

1. Biomechanics of the Heart

The heart is a four-chambered pump organ that provides systemic and pulmonary circulation and consists of the right and left atria and the right and left ventricles. These chambers are anatomically separated by the interatrial and interventricular septa, and unidirectional blood flow is ensured by the cardiac valves. The biomechanical function of the heart is primarily based on the contractile properties of the myocardial layer. In the cardiac wall structure, which consists of the endocardium, myocardium, epicardium, and pericardium, the myocardium is particularly responsible for active force generation. The propagation of electrical impulses originating from the sinoatrial node through the cardiac conduction system enables rhythmic contraction of the heart, thereby conferring the characteristics of a biological automatic pump (Kassab, 2006).

Myocardial muscle is a complex biological tissue exhibiting both active and passive mechanical properties. Active force generation occurs depending on

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muscle fiber length and intracellular calcium ion concentration, whereas passive mechanical properties are associated with the elastic components of the tissue and fiber organization. Therefore, the mechanical performance of the heart depends not only on contractile force but also on the interaction of ventricular geometry, loading conditions, and valvular function (Voorhees & Han, 2015).

One of the fundamental principles explaining the mechanical behavior of the heart wall is Laplace's law. This law describes the relationship between ventricular internal pressure, radius, and wall stress, demonstrating that an increase in radius, such as in ventricular dilation, leads to increased wall stress. Increased wall stress elevates myocardial energy consumption and may contribute to the development of heart failure (Karakoç & Naderi, 2020; Voorhees & Han, 2015).

The pressure–volume relationship plays an important role in evaluating the mechanical performance of the heart. The left ventricular pressure–volume cycle is a dynamic process that includes the phases of isovolumetric contraction, ejection, isovolumetric relaxation, and filling. The area enclosed by the curve represents the mechanical work performed by the ventricle and is considered a quantitative indicator of the heart's pump function (Voorhees & Han, 2015). Clinically, cardiac output and ejection fraction are fundamental parameters for assessing cardiac function. Cardiac output refers to the volume of blood pumped by the heart per minute, whereas ejection fraction indicates the efficiency of ventricular emptying (Karakoç & Naderi, 2020).

The biomechanics of the cardiac cycle is explained by the coordinated interaction of the systolic and diastolic phases. During systole, an increase in ventricular elastance leads to a rise in intraventricular pressure while torsional motion occurs in the left ventricle. This torsional deformation, resulting from the opposite rotational movements of the basal and apical segments, enhances ejection efficiency (Notomi et al., 2005). Diastole consists of active relaxation and passive filling processes. The release of elastic energy stored during systole in the early phase of diastole accelerates ventricular filling and improves the energetic efficiency of the heart (Zile, Baicu, & Gaasch, 2004). In this process, the combination of longitudinal, circumferential, and torsional motion components plays a critical role in the generation of stroke volume.

2. Biomechanics of Blood Vessels

The vascular system exhibits a multilayered mechanical structure composed of arteries, veins, and capillaries. All vessel types consist of three primary layers: the tunica intima, tunica media, and tunica adventitia. The intimal layer, formed by endothelial cells, plays a role in flow regulation and thrombosis control, whereas the media layer, composed of smooth muscle cells and elastic fibers, is responsible for regulating vascular tone. The adventitia supports vascular

integrity through its connective tissue components (Karakoç & Naderi, 2020; Kassab, 2006; Sünbülöğlu, 2007).

Arteries can withstand high pressures due to their thick walls and elastic properties, whereas veins function as capacitance vessels with thinner walls and a greater capacity for blood storage. Capillaries, with their single-layer endothelial structure, provide the microcirculatory environment in which substance exchange occurs (Hoskins et al., 2017).

The mechanical behavior of the vessel wall demonstrates viscoelastic–plastic properties. The organization of elastin and collagen fibers determines the stress–strain behavior of blood vessels, and the concept of compliance represents an important indicator of this biomechanical response. Compliance refers to the capacity for volume change in response to pressure variation, whereas elastance represents its inverse. In conditions such as aging and hypertension, increased vascular stiffness results in elevated systolic pressure and widening of pulse pressure (Hoskins et al., 2017; Karakoç & Naderi, 2020).

The propagation velocity of arterial pulse waves is an important biomechanical indicator of vascular elasticity; as arterial stiffness increases, pulse waves travel more rapidly. The early return of reflected pressure waves to the heart increases cardiac loading and contributes to the development of pathological processes (Hoskins et al., 2017). The venous system, with its large storage capacity, plays a critical role in maintaining the hemodynamic balance of circulation. Changes in venous tone can directly influence cardiac output, and venous return is affected by factors such as blood volume, autonomic nervous system activity, and gravity (Karakoç & Naderi, 2020).

3. Biomechanics of Blood Flow

Blood flow is determined by the interaction between pressure gradients, vascular resistance, and viscosity. Laminar flow is characterized by orderly and parallel flow lines, whereas turbulent flow is associated with irregular motion and increased energy loss. Turbulence generally occurs at vascular bifurcations or in regions of high pressure (Karakoç & Naderi, 2020).

Blood viscosity varies depending on hematocrit, temperature, and shear rate, and in the microcirculation, the apparent viscosity decreases as vessel diameter decreases due to the Fahraeus–Lindqvist effect (Erdal & Gençpınar, 2020). Endothelial cells are continuously exposed to shear stress, and these mechanical forces can influence vascular remodeling processes.

4. Biomechanics of the Lymphatic System

The lymphatic system is a network structure that plays an essential role in maintaining tissue fluid balance and supporting immune functions. Lymph flow is facilitated by the muscle pump, the valvular system, and pressure gradients,

while the high permeability of lymphatic capillaries facilitates the collection of interstitial fluid.

The viscosity of lymph fluid is lower than that of blood plasma, and flow generally occurs under low-pressure conditions. The pressure balance between the lymphatic and venous systems is critically important for maintaining body fluid homeostasis. In lymphedema, interstitial fluid accumulation leads to increased tissue pressure and alterations in tissue viscoelastic properties (Sünbuloğlu, 2007).

5. Biomechanical Alterations in Pathological Conditions

A substantial proportion of pathological changes occurring in the cardiovascular system are associated with biomechanical processes. Arterial stiffness, resulting from reduced vascular elasticity, leads to an increase in pulse wave velocity and, over the long term, increases left ventricular load, thereby predisposing to the development of heart failure (Kassab, 2006). Hypertension initiates vascular wall remodeling processes due to chronic mechanical loading and results in deterioration of elastic properties (Hoskins et al., 2017).

In atherosclerosis, plaque formation locally alters the mechanical properties of the vessel wall, and plaques with thin fibrous caps become more susceptible to rupture under high stress. In the development of aneurysms, heterogeneity in wall stress distribution is one of the primary factors determining the risk of rupture (Karakoç & Naderi, 2020).

In heart failure, ventricular wall stress increases and the effectiveness of the Frank–Starling mechanism decreases. Chronic pressure overload leads to ventricular hypertrophy, while over time diastolic filling becomes impaired and ejection fraction declines. During this process, remodeling mechanisms are activated within the myocardial tissue, and mechano-biological feedback systems influence cardiac function (Hoskins et al., 2017; Sünbuloğlu, 2007).

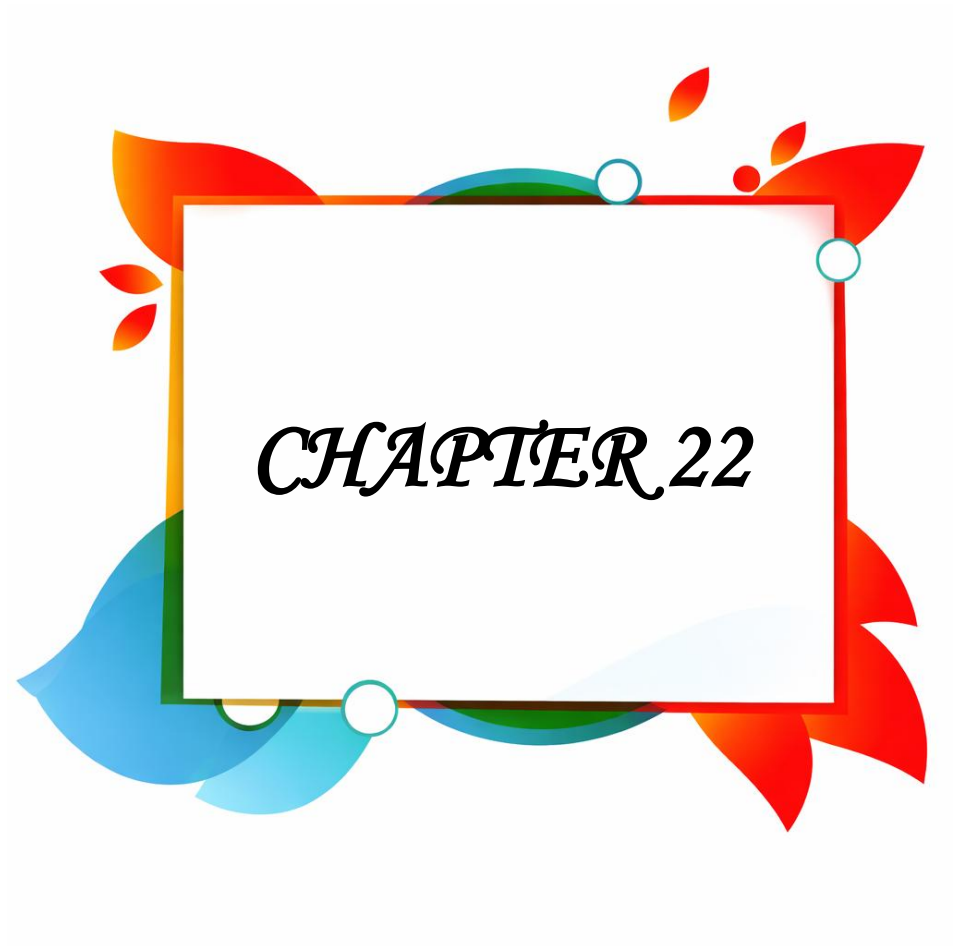
Conclusion

The biomechanics of the circulatory system is an extremely complex and multidimensional scientific field that requires the integrated evaluation of cardiac pump function, vascular elasticity, fluid dynamics, and lymphatic system interactions. The mechanical properties of the structures within this system and their dynamic interrelationships determine not only the maintenance of physiological circulation but also the emergence and progression of pathological processes. Therefore, a comprehensive understanding of biomechanical principles provides the fundamental scientific basis for explaining the pathophysiology of cardiovascular diseases, identifying risk factors, and developing effective clinical treatment approaches. Knowledge related to circulatory system biomechanics contributes significantly to disease prevention, the development of early diagnostic strategies, and the establishment of

individualized treatment plans. In recent years, rapidly advancing imaging technologies, computational biomechanical modeling methods, and biomaterial research have enabled more detailed analyses of the mechanical behavior of the cardiovascular system, and these developments are expected to make substantial contributions to personalized medicine applications in the future.

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Molecular Epidemiology and Regional Distribution of *Echinococcus Granulosus* Genotypes in Türkiye: A Review of Human and Animal Isolates

Saliha Zeynep Aydın¹

1. Introduction

Cystic echinococcosis is considered among the globally neglected parasitic zoonoses impacting both animal and public health. The World Health Organization (WHO) includes echinococcosis within the neglected tropical diseases requiring continuous surveillance and control programs due to its considerable medical, veterinary, and economic impact (Casulli, 2021). *Echinococcus granulosus sensu lato* has the broadest geographical distribution among *Echinococcus* species and accounts for most human CE infections across the world (Alvarez Rojas et al., 2014). The extensive public health impact of *E. granulosus* is closely related to its widespread occurrence in both definitive and intermediate hosts. The parasite maintains its life cycle between canids, primarily dogs, which serve as final hosts, and a variety of ungulate species, acting as intermediate hosts. Adult worms inhabit the small intestine of definitive hosts and release eggs that are shed into the environment with faeces. Under suitable conditions, these eggs can remain viable for prolonged periods. Following oral uptake of parasite eggs, the parasite develops into the metacestode stage and forms hydatid cysts, most commonly in the liver and lungs. Cyst growth is generally slow and may continue for months or years. Transmission is completed when infected organs containing viable cysts are consumed by a definitive host, allowing the parasite to mature into the adult stage and continue the cycle (Romig et al., 2017).

The classification of the *Echinococcus granulosus sensu lato* complex has changed considerably over time. Earlier studies regarded *E. granulosus* as a single species consisting of several strains. However, the use of molecular markers revealed substantial genetic variation within the complex and

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demonstrated differences among genotypes in their phylogenetic relationships, host preferences, and geographical distribution. As a result, the former *E. granulosus s.l.* complex is currently divided into five species: *E. granulosus sensu stricto*, *E. equinus*, *E. ortleppi*, *E. canadensis*, and *E. felidis* (Lymbery, 2017). Among the currently recognized species, *Echinococcus granulosus sensu stricto* is considered the most important from both epidemiological and public health perspectives.

Echinococcus granulosus sensu stricto is the most widespread species within the *E. granulosus sensu lato* complex and is responsible for the majority of human cystic echinococcosis cases worldwide. Molecular studies have demonstrated that G1 and G3 represent closely related lineages within *E. granulosus sensu stricto*, whereas G2 is no longer regarded as a valid genotype. The parasite is maintained primarily in dog–sheep transmission cycles, with sheep representing the principal intermediate host. However, *Echinococcus granulosus sensu stricto* exhibits relatively low host specificity and has been reported in a various domestic livestock, including goats, cattle, buffaloes, camels, pigs, and donkeys. In addition, various wildlife species may contribute to transmission in certain regions, and an independent sylvatic cycle has been documented. The wide host range and extensive geographical distribution of *Echinococcus granulosus sensu stricto* are considered important factors underlying its major public health and veterinary significance (Alvarez Rojas et al., 2014; Romig et al., 2017). *Echinococcus equinus* (G4), historically referred to as the horse-associated strain, is mainly maintained in transmission cycles involving equids, including horses, donkeys, and zebras, serving as intermediate hosts, whereas canids function as definitive hosts (Blutke et al., 2010). Unlike other members of the *E. granulosus sensu lato* complex, *E. equinus* is generally considered to have limited zoonotic significance (Blutke et al., 2010). Nevertheless, a small number of human infections have been reported, including cases from Türkiye and Uzbekistan (Kim et al., 2020; Macin et al., 2021).

Echinococcus ortleppi (G5), formerly known as the bovine strain, is currently recognized as a distinct species. Its life cycle is mainly maintained between dogs and cattle, with cattle representing the principal intermediate host. Although *E. ortleppi* has a worldwide distribution, it is generally reported less frequently than *E. granulosus sensu stricto*. The species has been identified in several regions of Africa, South America, and Europe, with reports from countries such as South Africa, Namibia, Zambia, and Brazil. Fertile cysts are most commonly detected in the lungs of cattle, but infections have also been described in goats and pigs. In addition, natural infections have been reported in some wild ungulates, including zebras and oryx antelopes. Human infections have been documented

from various parts of the world; however, they are uncommon. Phylogenetic studies have shown that *E. ortleppi* is closely related to the *E. canadensis* cluster (Romig et al., 2017).

Echinococcus canadensis (G6/G7), known as the camel and pig strains, is mainly maintained in dog–camel and dog–pig transmission cycles. While G6 is often seen in camels and G7 in pigs, both genotypes have also been reported in goats, sheep, and cattle. The parasite has a wide geographical distribution, extending across Europe, Central Asia, Africa, the Middle East, and South America. Infections have also been detected in several wildlife species, including wolves, wild boars, foxes, lions, and other wild ungulates (Romig et al., 2017). Human infections caused by G6/G7 have been reported from various endemic regions, and these genotypes account for approximately 11% of molecularly characterized human cystic echinococcosis cases worldwide (Alvarez Rojas et al., 2014). Additionally, G6 and G7, the *E. canadensis* complex includes the cervid-associated genotypes G8 and G10, which are predominantly associated with wildlife hosts (Šnábel, 2023). Recent findings have challenged the traditional distinction between the domestic G6/G7 and the sylvatic G8/G10 genotypes, as G6 has been identified in wolves and reindeer from the Altai and Yakutia in Russia, while G6/G7 has also been reported in reindeer (Šnábel et al., 2022). *Echinococcus felidis* (lion strain) is restricted to wildlife transmission cycles and has been reported exclusively in sub-Saharan Africa. To date, no human cases have been reported (Casulli et al., 2022). Since morphological characteristics alone are insufficient for reliable genotype discrimination, molecular approaches have become the primary tools for the characterization of *E. granulosus* isolates. Among the markers used, mitochondrial gene regions such as *cox1*, *nad1*, *nad5*, *rrnS*, 12S rRNA, and *atp6* are the most commonly analyzed due to their ability to distinguish closely related genotypes and provide information on their phylogenetic relationships (Gholami et al., 2012).

This review aims to summarize PCR-based genotype studies conducted in Türkiye and to evaluate the distribution of genotypes and species associated with cystic echinococcosis infections. The secondary aim of this review is to evaluate the epidemiological and practical importance of strain distribution in different geographical regions, particularly in terms of transmission dynamics and region-specific control strategies.

2. Materials and Methods

This review was conducted using studies investigating molecular characterization and genotype distribution of *Echinococcus granulosus sensu lato* in Türkiye. Literature searches were performed using Google Scholar. The database search incorporated combinations of the following keywords using

Boolean operators AND and OR: (“*Echinococcus granulosus*” OR hydatid cyst OR cystic echinococcosis) AND (genotype OR molecular characterization OR PCR OR sequencing OR *cox1* OR *nad1*) AND (Türkiye OR Turkey). Additional searches were also performed using some province-based keywords including Erzurum, Elazığ, Van, Şanlıurfa, Ankara, Konya, Samsun, İzmir, Aydın, Manisa and other regions where molecular studies were available.

3. Results

3.1. Eastern Anatolia Region

3.1.1. Erzurum

In Erzurum Province, 220 cattle-derived hydatid cyst isolates were analysed using PCR amplification of the 12S rRNA gene and sequencing of the mitochondrial *cox1* gene. Among the examined isolates, 147 were assigned to *Echinococcus granulosus sensu stricto* (G1–G3 complex). Further *cox1* sequence analysis confirmed the presence of G1 and G3 genotypes, with G1 representing the leading genotype among the sequenced isolates, whereas 7 isolates were identified as G3 (Simsek et al., 2010).

Hydatid cyst isolates obtained from sheep slaughtered in Erzurum Province and originating from different provinces of Türkiye, including Erzurum, Şanlıurfa, Muş, Van, Konya, Afyonkarahisar, Iğdır, Yozgat, Erzincan, Amasya, and Elazığ were analysed using PCR amplification of the mitochondrial 12S rRNA and *cox1* gene regions. Of the 351 cyst samples examined, 315 yielded positive results by 12S rRNA PCR. Sequence analysis of 70 selected isolates identified all samples as *Echinococcus granulosus sensu stricto*. Among these isolates, 54 (77.1%) were classified as G1 and 16 (22.9%) as G3 (Avcioglu et al., 2022).

In addition, Hydatid cyst materials obtained from 61 patients residing in Erzurum, Erzincan, Ağrı, Ardahan, Iğdır, Kars, and Bayburt between 2019 and 2023 were analysed. Molecular characterization was performed using PCR assays targeting the mitochondrial 12S rRNA and *mt-COX1* gene regions. Of the 61 cyst samples examined, 37 yielded positive results by 12S rRNA PCR, while an additional 11 samples were identified through *mt-COX1* PCR, resulting in a total of 48 (78.7%) PCR-positive samples. Sequence analysis of selected *mt-COX1* amplicons identified all sequenced isolates as *Echinococcus granulosus sensu stricto* (G1, G3) (Yılmaz et al., 2025).

In another study, a total of 446 faecal samples collected from stray dogs in Erzurum analysed. Taeniid eggs were detected microscopically in 119 samples. Molecular analysis targeting the 12S rRNA gene identified *Echinococcus* spp. in

63 samples. Species-specific 12S rRNA PCR detected *E. granulosus sensu stricto* (G1/G3) in 41 samples and *E. multilocularis* in 16 samples. Amplification and sequencing of the mitochondrial *cox1* gene further identified *E. equinus* (G4) in three samples, *E. ortleppi* (G5) in one sample, and *E. canadensis* (G6/G7) in three samples. The detection of *E. ortleppi* (G5) represented the first molecular record of this species in Türkiye reported in the study (Avcioglu et al., 2021).

A further molecular investigation performed on a gray wolf (*Canis lupus*) found near Erzurum Province was examined for *Echinococcus* spp. infection. Morphological examination identified a mixed infection consisting of *E. granulosus sensu lato* and *E. multilocularis*, with parasite burdens of 62,720 and 49,280 worms, respectively. Molecular characterization was performed using PCR targeting the mitochondrial *COI* and 12S rRNA gene regions, followed by sequence analysis. The isolates were identified as *E. granulosus sensu stricto* (G1-G3) and *E. multilocularis*. This study representing the first documented co-detection of these parasites in a gray wolf in Türkiye and highlighted the possible contribution of wildlife hosts to parasite circulation in Erzurum (Akyuz et al., 2024).

Overall, 250 equids, (206 donkeys and 44 horses) originating from different districts of Erzurum Province, were examined by ELISA using antigen B derived from sheep hydatid cyst fluid. Anti-*Echinococcus granulosus* antibodies were found in 78 animals (31.2%). Seropositivity was identified in 69 of 206 donkeys (33.5%) and 9 of 44 horses (20.4%). Among donkeys, the highest seroprevalence was recorded in Çat (48.7%), followed by Horasan (41.5%), Olur (25.0%), and Oltu (18.4%). In horses, seropositivity rates were 23.1% in Yakutiye, 18.2% in Aziziye, and 14.2% in Horasan (Balkaya & Simsek, 2011).

3.1.2. Elazığ

In a study including paediatric cystic echinococcosis cases from Elazığ and Muş, molecular characterisation was performed on 17 hydatid cyst isolates using PCR amplification and sequence examination of the mitochondrial *cox1* gene. Sixteen isolates were confirmed as *Echinococcus granulosus sensu stricto*, while 1 isolate was classified as *E. canadensis* (G6/G7). The G6/G7 isolate was recovered from a young patient from Elazığ, representing the first reported detection of this genotype in paediatric cystic echinococcosis cases in Türkiye (Bakal et al., 2021).

Molecular genotyping was performed on 41 hydatid cyst isolates recovered from sheep slaughtered in Bingöl and Elazığ. PCR amplification and sequencing targeted the mitochondrial *mt-COI* and *mt-Nad5* regions together with the nuclear *eflα* marker. All 41 isolates yielded *mt-COI* sequence data and were

assigned to *Echinococcus granulosus sensu stricto* (G1/G3). *mt-Nad5* sequences were obtained from 40 isolates, as one sample did not produce a sequence suitable for further analysis. Among these isolates, 38 were classified as G1 and two as G3. Sequence information from the *ef1a* region was available for 23 isolates (Gunyakti Kilinc et al., 2025).

Molecular characterization was performed on 85 hydatid cyst isolates obtained from 66 cattle and 19 sheep in Elazığ Province. Genomic DNA extracted from germinal membranes was analysed by PCR and sequencing of the mitochondrial *mt-COI* gene region. Sequence data identified 84 isolates as *Echinococcus granulosus sensu stricto*, whereas one sheep isolate was classified as *Echinococcus canadensis* (G6/G7). The *E. canadensis* genotype was confirmed by analysis of an additional *mt-COI* fragment. This finding represented the first molecular detection of *E. canadensis* (G6/G7) in sheep in Türkiye (Mehmood et al., 2020).

A molecular study conducted on cattle in Elazığ Province analyzed 40 hydatid cyst isolates recovered from the lungs of slaughtered animals. Sequence analysis of the mitochondrial *mt-COI* gene identified 39 isolates as *Echinococcus granulosus sensu stricto* (G1–G3) and one isolate as *Echinococcus canadensis* (G6/G7). The G6/G7 isolate represented the first report of *E. canadensis* detected in cattle in Türkiye (Kesik et al., 2021).

Another retrospective investigation covering 52 equine necropsies performed in Elazığ included molecular examination of CE detected in one Arabian horse and two donkeys. Germinal membrane samples obtained from hepatic and pulmonary cysts were analysed by PCR amplification and sequencing of the mitochondrial *mt-COI* gene region. Sequence data generated from 17 cyst isolates identified all samples as *Echinococcus equinus* (G4) (Celik et al., 2025).

3.1.3. Malatya

Molecular characterization of hydatid cysts was carried out in a Turkish mouflon (*Ovis gmelinii anatolica*) from Malatya Province. Specimens collected and analysed using PCR amplification and sequencing of a 446 bp fragment of the mitochondrial *mt-COI* gene. Sequence analysis identified all three liver cyst isolates as *Echinococcus granulosus sensu stricto* corresponding to the G1 genotype (Simsek & Eroksuz, 2009).

3.1.4. Kars

A molecular study investigating the genetic variability of *Echinococcus granulosus* in livestock from Türkiye analyzed 112 hydatid cyst isolates, including 12 cattle isolates originating from Kars Province. Genotyping based on partial mitochondrial cytochrome c oxidase subunit 1 (cox1) sequencing identified G1 and G3 genotypes among the Kars isolates. Nine cattle isolates belonged to the G1 genotype, whereas three isolates were identified as G3 (Vural et al., 2008).

A nationwide molecular study included seven human hydatid cyst isolates originating from Eastern Anatolia, comprising samples from Ağrı, Bitlis, Hakkari, Kars, Malatya, and Van. DNA isolated from the cyst material was used for PCR targeting the cox1 gene. All Eastern Anatolian samples were classified as *Echinococcus granulosus sensu stricto* and were distributed among haplotypes belonging to the major G1 and G3 lineages detected in the study population (Örsten et al., 2018).

3.1.5 Bingöl

A molecular survey of wild boars collected in Bingöl Province examined cystic metacestode infections using mitochondrial cox1 sequencing. Necropsy of 23 wild boars yielded nine hydatid cyst isolates. Among the hydatid cyst samples, cox1 amplification was successful in eight isolates. Sequence analysis assigned seven isolates to *Echinococcus granulosus sensu stricto* (G1/G3) and one isolate to *Echinococcus canadensis* (G6/G7). To differentiate G1 and G3 within *E. granulosus sensu stricto*, the positive samples were further analysed using a 759 bp fragment of the mitochondrial nad5 gene. Based on nad5 sequence data, five isolates belonged to the G1 genotype and two to the G3 genotype (Gunyakti Kilinc et al., 2024).

A grey wolf found in Bingöl Province was examined for adult *Echinococcus* spp. infection. Molecular characterization based on mitochondrial cox1 sequencing was performed on 9 adult worms recovered from the intestine. Six isolates were identified as *Echinococcus equinus*, whereas 3 isolates belonged to *Echinococcus canadensis* (G6/G7). This represented the first detection of a mixed *E. equinus* and *E. canadensis* (G6/G7) infection in a grey wolf in Türkiye (Gunyakti Kilinc et al., 2023).

In a study investigating 41 cysts collected from sheep in Elazığ and Bingöl provinces, mitochondrial mt-CO, mt-Nad5 and nuclear efl α gene regions were analysed by PCR and sequencing. mt-CO1 sequence data assigned all isolates to *Echinococcus granulosus sensu stricto* (G1/G3). Genotyping with the mt-Nad5

marker identified 38 isolates as G1 and 2 isolates as G3, while one sample could not be sequenced successfully for this locus (Gunyakti Kilinc et al., 2025).

3.2. Southeastern Anatolia Region

3.2.1. Şanlıurfa

A molecular investigation conducted in Şanlıurfa Province evaluated hydatid cyst isolates recovered from humans and livestock. The dataset consisted of 197 cyst specimens collected from 40 humans, 140 sheep and 17 cattle. After microscopic examination, 33 human and 80 sheep cysts were identified as fertile. Samples with sufficient protoscolex content were analysed by PCR-RFLP targeting the ribosomal *rDNA-ITS1* region using the restriction enzymes *AluI*, *RsaI*, and *TaqI*. A total of 60 isolates, including 13 human and 47 sheep samples, were successfully genotyped. All analysed isolates showed the same restriction pattern and were identified as *Echinococcus granulosus sensu stricto*, corresponding to the G1 genotype (Günbegi Çalışkan & Yıldız Zeyrek, 2024).

In addition, A molecular study investigating 46 human cystic echinococcosis isolates from different regions of Türkiye included four isolates originating from Şanlıurfa Province. DNA extracted from cyst material was analysed by PCR amplification and sequencing of an 827 bp fragment of the mitochondrial *cox1* gene. Haplotype network analysis assigned one isolate to the dominant *Echinococcus granulosus sensu stricto* (G1/G3 complex) cluster, two isolates to a G1-associated cluster, and one isolate to a G3-associated cluster (Orsten et al., 2018).

3.2.2. Kilis

Sheep-derived hydatid cyst samples collected in Kilis Province were examined by molecular methods. The dataset consisted of 28 cyst isolates obtained from 19 animals, including 20 fertile cysts. PCR amplification targeting the mitochondrial *12S rRNA* region and the ribosomal *rrnS* gene produced positive results for all isolates, placing the samples within *Echinococcus granulosus sensu stricto* (G1-G3 complex). Two isolates from different sheep were subsequently selected for PCR amplification and sequencing of the mitochondrial *mt-COI* gene. Comparison of the obtained *mt-COI* sequences with reference data demonstrated 99% similarity to sequences belonging to *Echinococcus granulosus sensu stricto* (G1 genotype) (Utuk et al., 2012). *Echinococcus*

3.2.3. Siirt

Hydatid cyst material collected from sheep and goats in Siirt Province was examined by PCR amplification and sequencing of the mitochondrial *mt-COI*

gene region. Sequence data were obtained from 76 of the 138 cyst samples analysed. All 61 sheep isolates were assigned to *Echinococcus granulosus sensu stricto* (G1/G3 complex). Among 13 goat isolates, 7 belonged to *E. granulosus sensu stricto* (G1/G3 complex), whereas 6 were identified as *Echinococcus canadensis* (G6/G7) (Selcuk et al., 2024).

In another study, molecular analysis of 46 human hydatid cyst isolates collected from different regions of Türkiye, 13 samples originated from Southeastern Anatolia. All isolates were characterized as *Echinococcus granulosus* G1 based on mitochondrial CO1 gene sequencing (Ergin et al., 2010).

3.3. Aegean Region

3.3.1. İzmir

A molecular study carried out in western Türkiye included hydatid cyst isolates from İzmir Province, covering İzmir city centre together with the districts of Menemen, Bergama and Selçuk. PCR amplification and sequencing of mitochondrial *cox1*, *atp6*, *nad1* and *rrnS* gene regions were used for genotype identification. Thirteen isolates were classified as *Echinococcus granulosus sensu stricto*. One sheep isolates from Bergama corresponded to the G3 genotype, while another sheep isolate from İzmir showed a G1/G3 genotype profile. Three isolates were identified as *Echinococcus canadensis* (G7), including two sheep isolates from İzmir and one human isolate from Selçuk (Šnábel et al., 2009).

3.3.2. Aydın

20 human hydatid cyst isolates obtained from surgically treated cases in Aydın Province between 2013 and 2016 were examined by PCR amplification and sequencing of the mitochondrial *cox1* gene. Fifteen isolates (75%) were identified as *Echinococcus granulosus sensu stricto* (G1), while 5 isolates (25%) associated to *Echinococcus canadensis* (G6/7 genotype) (Oral Babaoğlu et al., 2018).

Another survey was conducted in owned dogs from Aydın Province and its surrounding districts. Faecal samples collected from 100 dogs were examined by flotation and PCR targeting a 446 bp fragment of the mitochondrial *cox1* gene. *Echinococcus granulosus* DNA was detected in 1 sample (1%) (Boğa Kuru et al., 2013)

3.3.3. Afyon

A study conducted in Afyonkarahisar investigated hydatid cyst isolates originating from cattle, buffaloes, sheep, and goats originating from the central district and surrounding districts. Hydatid cyst material was collected from 258

infected animals, comprising 119 cattle, 71 sheep, 65 goats, and 3 buffaloes. Molecular analyses were performed on 78 isolates, including samples from goats (n=30), sheep (n=26), cattle (n=19), and buffaloes (n=3). PCR-RFLP analysis of the ND1 gene region showed no polymorphism among the examined isolates. Sequencing of the mt-COX1 gene identified all analyzed samples as *Echinococcus granulosus sensu stricto* (G1 genotype) (Kartal et al., 2020).

3.3.4. Manisa

In the study by Šnábel et al. (2009), one sheep isolates and one human isolate from Manisa were analysed by sequencing of mitochondrial *cox1*, *atp6*, *nad1* and *rrnS* gene regions. Both isolates were assigned to *Echinococcus granulosus sensu stricto*. A later study examined 18 hydatid cyst isolates collected from cattle slaughtered in Manisa Province between 2010 and 2012. Sixteen cysts originated from the liver and two from the lungs. Molecular characterization relied on PCR amplification and sequencing of the mitochondrial *cox1* and *nad1* gene regions. All 18 isolates were identified as *Echinococcus granulosus sensu stricto* (G1-G3 complex) (Šnábel et al., 2009; Altıntaş et al., 2013).

3.3.5. Denizli and Uşak

Denizli and Uşak Provinces were each represented by a single sheep isolate in a molecular study conducted in western Turkey. Molecular characterization achieved through PCR and sequencing of mitochondrial *cox1*, *atp6*, *nad1* and *rrnS* gene regions. Both isolates were assigned to *Echinococcus granulosus sensu stricto*. One isolate originated from Denizli and one from Uşak (Šnábel et al., 2009).

3.4. Marmara Region

3.4.1. Istanbul

A molecular survey investigated the presence of *Echinococcus granulosus* in 110 fecal samples collected from stray dogs in seven districts of Istanbul, including Ümraniye, Beykoz, Çekmeköy, Sancaktepe, Sultanbeyli, Ataşehir, and Üsküdar. Molecular characterization based on mitochondrial CO1 gene analysis identified *Echinococcus granulosus sensu stricto* in two samples (2/110). The positive samples originated from Beykoz and Sultanbeyli districts and were classified as G1 genotype. Phylogenetic analysis classified both isolates within the *E. granulosus sensu stricto* (G1-G3) complex (Akil et al., 2026).

3.4.2. Edirne

The first molecular data from the Thrace Region were reported by Eryıldız and Şakru (2012). In that study, 58 hydatid cyst isolates collected in Edirne

including 42 human, 13 cattle and 3 sheep isolates. Molecular characterization was performed using PCR-RFLP analysis of the ITS1 region, PCR-SSCP targeting the mitochondrial *nad1* gene, and sequence analysis of the mitochondrial *cox1* and *nad1* gene regions. 13 representative isolates were selected for sequencing. Genotyping results identified two distinct genotypes, G1 and G7. Among the 58 isolates examined, 57 were classified as *Echinococcus granulosus sensu stricto* (G1 genotype), whereas one human isolate was identified as the G7 genotype. The G1 genotype was detected in human, cattle and sheep isolates, while the G7 genotype was found in a single human isolate (Eryıldız & Şakru, 2012).

In a molecular study undertaken in Edirne, 75 hydatid cyst samples obtained from humans, cattle and sheep were analysed. Initial genotyping based on the 12S rRNA gene placed all isolates within the *Echinococcus granulosus sensu stricto* (G1-G3) complex. 17 representative isolates were characterized by sequencing of the mt-CO1 and mt-ND1 gene regions. G1, G3 genotypes were detected among the sequenced samples as well as isolates that were reported as G2 according to the classification system used in the original study (Tarladaçalışır et al., 2022).

Three human hydatid cyst isolates originating from the Marmara Region were included in a molecular study evaluating *Echinococcus granulosus* genotypes in Türkiye. All analyzed isolates were identified as the G1 genotype (Ergin et al., 2010).

3.4.3. Tekirdağ

Hydatid cyst samples collected from sheep originating from Tekirdağ Province were included in a molecular genotyping study. A total of 18 isolates were examined by PCR and mitochondrial *cox1* gene sequencing. The analysed samples were assigned to the *Echinococcus granulosus sensu stricto* (G1 genotype) (Vural et al., 2008).

3.5. Central Anatolia Region

3.5.1. Kayseri

A molecular characterization study conducted in Kayseri evaluated 39 hydatid cyst isolates obtained from 25 humans, 8 cattle, 4 sheep, and 2 goats. Genotyping was carried out using PCR amplification and sequence analysis of the mitochondrial COX1 gene. All examined isolates were identified as *Echinococcus granulosus sensu stricto* (G1 genotype) (Erdoğan et al., 2017).

3.5.2. Yozgat

In a molecular study investigating livestock-derived hydatid cyst isolates from different regions of Türkiye, 26 sheep samples from Yozgat Province also included. Samples were analysed using mitochondrial *cox1* gene sequencing. The examined isolates were classified within the *Echinococcus Granulosus* (G1 genotype), and no G3 associated isolates were detected among the analysed samples (Vural et al., 2008).

3.5.3. Kırıkkale

A molecular genotyping study conducted in Kırıkkale Province investigated hydatid cyst isolates obtained from cattle slaughtered in the region. A total of 20 cyst isolates collected from the liver (n=15) and lungs (n=5) were analysed. Molecular characterization was performed by PCR amplification of the mitochondrial 12S rRNA and cytochrome c oxidase subunit 1 (COI) gene regions, followed by COI sequence analysis. All examined isolates were classified within the *Echinococcus granulosus sensu stricto* (G1–G3) complex according to the sequencing results (Gökpınar et al., 2017).

3.5.4. Konya

In Konya Province, molecular data were obtained from 83 hydatid cyst isolates collected from cattle (n = 57) and humans (n = 26). The mitochondrial *cox1* gene region was used for molecular characterization. Among the examined isolates, 82 were assigned to *Echinococcus granulosus sensu stricto* (G1–G3 complex), while a single human isolate was identified as *Echinococcus equinus* (G4). This study provided the first molecular evidence of *E. equinus* in a human case from Turkey (Macin et al., 2021).

3.5.5. Aksaray

Aksaray Province was represented by a molecular study based on samples obtained from 60 patients diagnosed with cystic echinococcosis at a tertiary care hospital. Analysis of the mitochondrial *cox1* gene showed that 25 isolates belonged to the G1 genotype and 18 to the G3 genotype of *Echinococcus granulosus sensu stricto*. The original study also classified 10 isolates as G2 according to the genotyping scheme used by the authors. In addition, 4 isolates were assigned to *Echinococcus equinus* and 3 to *Echinococcus ortleppi*. The detection of *E. ortleppi* in human clinical samples constituted the first report of this species in human cystic echinococcosis cases from Turkey (Eroğlu & Ordu, 2023).

A molecular study included 26 human cystic echinococcosis isolates originating from Central Anatolia, comprising samples from Ankara (n=16),

Aksaray (n=2), Çankırı (n=2), Eskişehir (n=1), Karaman (n=1), Kırıkkale (n=1), Sivas (n=1), and Yozgat (n=2). Sequencing of an 827-bp fragment of the mitochondrial *cox1* gene demonstrated that all isolates belonged to *Echinococcus granulosus sensu stricto*. Among the Central Anatolian isolates, 15 were included in the dominant *E. granulosus* s.s. cluster, 7 were grouped within the G1-associated cluster, and 4 were assigned to the G3-associated cluster (Örsten et al., 2018).

3.5.6. Ankara

A multicentre molecular study evaluated hydatid cyst isolates collected from different provinces of Turkey, including Ankara, Ordu, Adana and Mersin. In Ankara, cystic echinococcosis was detected in 35 of 702 cattle and 9 of 390 sheep examined at slaughterhouses. Following the exclusion of calcified and unsuitable cysts, 13 isolates were included in the molecular analyses. Genotyping was based on mitochondrial *cox1* sequences, while *mt-nad5* analysis was used to verify G3 isolates. Of the analysed samples, 8 originated from cattle and 5 from sheep. The cattle isolates comprised 6 G1-associated cluster isolates and 3 G3-associated cluster isolates, whereas among sheep isolates, 2 belonged to the G1-associated cluster and 2 to the G3-associated cluster of *Echinococcus granulosus sensu stricto* (Cengiz & Gonenç, 2020).

3.6. Black Sea Region

In Samsun, Ordu and Amasya provinces, a molecular investigation was conducted on hydration cyst isolates collected from slaughtered water buffaloes. Hydatid cysts were detected in 17 of 166 examined animals, and molecular characterization was carried out on 9 isolates using mitochondrial *cox1* sequences. Among the analysed isolates, 6 corresponded to the G1 genotype of *Echinococcus granulosus sensu stricto*, whereas 3 showed sequence variants within the G1-G3 complex. No genotypes other than *E. granulosus sensu stricto* were reported in the analysed buffalo samples (Beyhan & Umur, 2011).

A multicentre molecular characterization study evaluated hydatid cyst isolates collected from different provinces of Turkey, including Ankara, Ordu, Adana and Mersin. In Ordu, cystic echinococcosis was detected in 49 sheep, whereas no cases were recorded among cattle, out of a total of 126 examined animals. Following the exclusion of calcified and unsuitable cysts, 24 sheep derived isolates were analysed using mitochondrial *cox1* sequences, and *nad5* analysis was performed to confirm G3 isolates. Among the genotyped samples, 20 belonged G1 genotype and 4 to the G3 genotype of *Echinococcus granulosus sensu stricto*. (Cengiz & Gonenç, 2020).

A molecular study analysed human cystic echinococcosis isolates originating from the Black Sea Region, including Çorum (n=3) and Tokat (n=1). DNA extracted from hydatid cyst material was used for PCR amplification and sequencing of the mitochondrial *cox1* gene. All analysed isolates were identified as *Echinococcus granulosus sensu stricto*. Haplotype network analysis showed that 3 isolates were included in the dominant *E. granulosus s.s.* cluster, whereas 1 isolate was grouped within the G1-associated cluster. No isolates from the Black Sea Region were assigned to the G3-associated cluster. (Orsten et al., 2018).

3.7. Akdeniz

3.7.1. Adana

A Molecular analysis conducted in Adana Province examined 20 hydatid cyst specimens obtained from human patients. Genotyping analyses identified all examined isolates as the *Echinococcus granulosus sensu stricto* (G1 genotype) (Eroğlu et al., 2016). Another molecular investigation examined hydatid cyst isolates collected from several provinces in Türkiye, including Adana. Among 315 animals inspected in Adana, cystic echinococcosis was recorded in 10 sheep and 6 cattle. Following the exclusion of samples which are unsuitable for DNA analysis, 7 fertile sheep isolates were characterised using sequencing of the mitochondrial *cox1* sequences, and *mt-nad5* analysis was applied to confirm G3 identifications. Genotyping identified 3 isolates as the G1 genotype and 4 isolates as the G3 genotype of *Echinococcus granulosus sensu stricto* (Cengiz & Gonenç, 2020).

3.7.2. Mersin

A multicentre molecular study evaluated hydatid cyst isolates collected from Ankara, Ordu, Adana and Mersin. Among the animals examined in Mersin, cystic echinococcosis was detected in 7 of 30 cattle and 3 of 368 sheep. After unsuitable cysts were excluded, 3 isolates were available for molecular characterization. Molecular characterization was performed using mitochondrial *cox1* sequences, while *mt-nad5* analysis was used to confirm G3 isolates. 2 isolates were assigned to the G1 associated cluster and 1 isolate to the G3 associated cluster of *Echinococcus granulosus sensu stricto*. The G1 isolates originated from a cattle lung cyst and a sheep lung cyst, whereas the G3 isolate was obtained from a sheep liver cyst (Cengiz & Gonenç, 2020).

3.7.3. Hatay

In Hatay Province, a molecular investigation was carried out on human hydatid cyst samples collected from patients diagnosed with cystic echinococcosis. Of the 35 cyst samples collected from 31 patients, 11 fertile cysts

containing protoscoleces were available for molecular analysis. All of the isolates were analysed according to the mitochondrial *cox1* sequences and placed within the *Echinococcus granulosus sensu stricto* (G1–G3). (Hamamcı et al., 2023).

4. Discussion

Although *E. granulosus sensu stricto* (G1/G3) was the predominant species identified in both human and animal hosts in Türkiye, this review also demonstrated the presence of *E. canadensis* (G6/G7), *E. equinus* (G4) and *E. ortleppi* (G5) in different geographical regions and host species. G6/G7 isolates were reported from humans, sheep, cattle, goats and dogs, whereas G4 was detected not only in horses and donkeys, its typical hosts, but also in dogs and human cases. Likewise, G5 was identified in both dogs and humans. In addition, molecular studies conducted in wildlife hosts identified *E. granulosus sensu stricto*, *E. canadensis* (G6/G7) and *E. equinus* in grey wolves, wild boars and a Turkish mouflon, suggesting that wildlife can also contribute to parasite circulation in some regions of the country (Simsek & Eroksuz, 2009; Akyüz et al., 2024; Gunyakti Kilinc et al., 2023; Gunyakti Kilinc et al., 2024). Taken together, these findings indicate that the epidemiology of cystic echinococcosis in Türkiye is more diverse than would be inferred from human cases alone and cannot be explained basically by the classical sheep-dog transmission cycle.

The identification of circulating species and genotypes is important not only for describing parasite diversity but also for understanding transmission patterns and host associations. Knowledge of genotype distribution can help identify reservoir hosts, clarify transmission routes and support the implementation of region-specific control measures. It may also be relevant for the development and evaluation of diagnostic tools, vaccines and other preventive approaches. Although the EG95 vaccine has shown high levels of protection against *E. granulosus sensu stricto*, antigenic differences reported among other members of the complex suggest that genetic variation should be considered when assessing vaccine performance in endemic areas (Borhani et al., 2024). In addition, differences in host adaptation and developmental characteristics may influence parasite transmission and could potentially affect the optimal timing and frequency of interventions such as dog deworming programmes (Casulli et al., 2022). Although genotype identification does not currently alter the routine clinical management of cystic echinococcosis, further molecular studies involving a wider range of host species are needed to better understand the epidemiological and biological significance of less frequently reported species such as *E. canadensis*, *E. equinus* and *E. ortleppi*. Such information may contribute to the development of more effective and locally adapted control strategies within a One Health framework.

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