REVIEW ARTICLE

TISSUE ENGINEERING IN PERIODONTAL REGENRATION: A REVIEW

Rahul Kesharwani[1], Ankita Dwivedi[2], Deepak Chopra[3], Mushahida Anjum[4], Vivek Tripathi[5], Sujan Kambhapati[6]

1. Post Graduate Student, Dental College And Hospital, Azamgarh
2. Post Graduate Student, Dental College And Hospital, Azamgarh.
3. Professor And Head, Dental College And Hospital, Azamgarh
4. Post Graduate Student, Dental College And Hospital, Azamgarh
5. Senior Lecturer, Dental College And Hospital, Azamgarh
6. Professor and Head, Department of Prosthodontics, Dental College Azamgarh

ABSTRACT

The tissue engineering as favourable application has been to harness its ability to make use of selected and primed cells together with an appropriate mix of regulatory factors, to allow growth and specialization of cells and matrix. Studies confirmed that the periodontal regeneration with the use of combination of tissue engineered products with an osteoconductive matrix improve the beneficial effect of these materials by accelerating cellular in growth and revascularization of the wound site. Studies have suggested the use of rh Platelet-derived growth factor+beta tricalcium phosphate for regeneration of the periodontal attachment apparatus in combination with collagen membranes as an acceptable alternative to connective tissue graft for covering gingival recession defects. Studies concluded that growth factors promote true regeneration of the periodontal attachment apparatus and the use of combination protein therapeutics which is commercially available can provide more expected, faster, less invasive, less traumatic, and efficient outcome for the patient.

Keywords: Tissue Engineering, Cell, Periodontal Regeneration, Recombinant Therapeutics.

INTRODUCTION

Periodontal treatment is to completely periodontium including cementum, periodontal ligament, and alveolar bone lost due to periodontal disease or trauma. Quality of periodontal regeneration to a complete healing of the periodontal tissues in both height and function, that is, the formation of alveolar bone, connective attachment through collagen fibers functionally oriented on the newly formed cementum.[1] In the past few decades, many attempts have been made to unravel the "magic filler" material that could result in new clinical and histological attachment, but have only culminated in healing by repair. Periodontal repair refers to curing that does not allow the original morphological nor functional restoration of the tissue, considered as non-functional scarring.Regeneration of the periodontal tissues is a complex phenomenon requiring

Corresponding Author: Dr Rahul Kesharwani Email: drrahulkesharwani@gmail.com

relationship between various processes in a timely manner. Tissue engineering was proposed as a possible technique for regenerating lost periodontal tissues by Langer and colleagues in 1993.[2] Tissue engineering is an interdisciplinary field that applies principles and methods of engineering and the life sciences towards the development of biological substitutes that restore, maintain, and improve the function of damaged tissues and organs.[3]The aim of tissue engineering is to promote healing and ideally, true regeneration of a tissue's structure and function, , more quickly, more predictably, less invasively, and more qualitatively than allowed by previous passive techniques.[4,5]

TISSUE ENGINEERING MOVE TOWARDS

The tissue engineering approach to bone and periodontal regeneration combines three important requisites to enhance regeneration.

- Scaffolds
- Signals
- Stem/Progenitor cells.[6]

This notion is often represented as a triangle, indicating that by combining the three key essentials tissue regeneration can often be accomplished Figure 1.

Scaffolds

Scaffold provide a 3D substratum on to which the cells can proliferate and migrate, produce a matrix and form a functional tissue with a desired shape. A suitable bioactive 3D scaffold for the promotion of cellular proliferation and differentiation is critical in periodontal tissue engineering.

Scaffold plays role in tissue regeneration process:[7]

1. Provide physical support for the healing area.

2. Serve as a barrier to restrict cellular migration in a selective manner. This is best exemplified by the principles of guided tissue regeneration and guided bone regeneration where nonresorbable polytetrafluoroethylene and resorbable polylactate, polyglyolic acid and calcium sulfate are used.

3. To serve as a scaffold for cellular migration and proliferation. Examples include collagen matrix. Potentially, this scaffold can be further enhanced by selectively defining the types of cells permitted to attach to and proliferate on this matrix with the additions of adhesins and⁄or integrins.

4. Before its absorption, a scaffold can serve as a matrix for exogenous and endogenous cell adhesion and thus facilitates and regulates certain cellular processes, including mitosis, synthesis and migration.

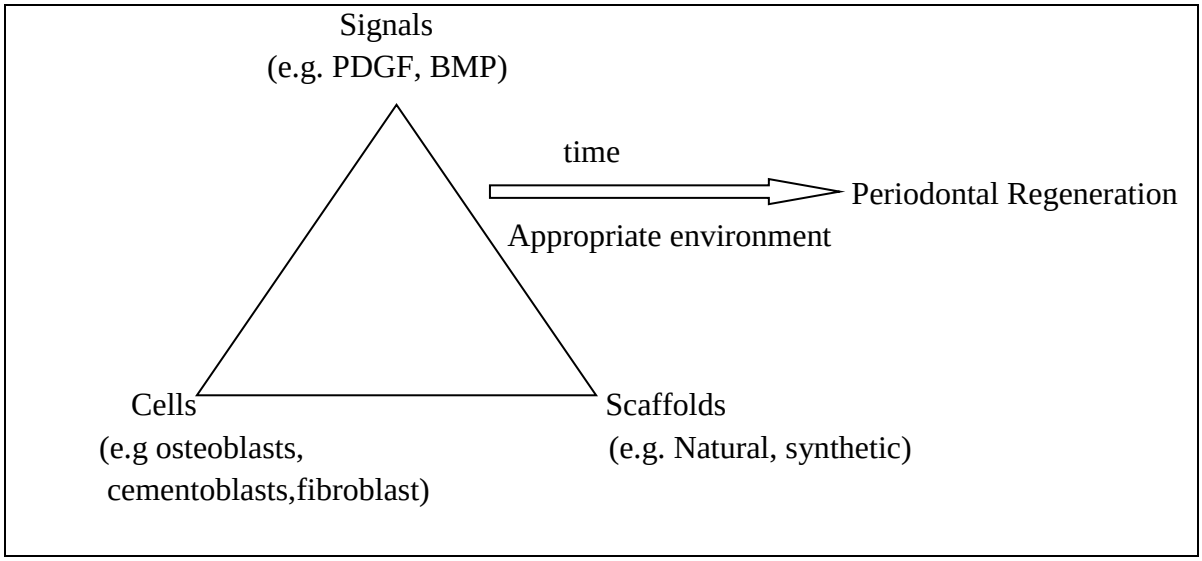

Figure1: determinants of tissue engineering in periodontology

Biomaterials used as scaffolds in tissue engineering are classified into two broad categories [Table 1].

- Naturally derived.
- Synthetic.

Biomaterials used as scaffolds

Table 1: biomaterial used for the rationale of periodontal regeneration

Naturally derived	**Synthetic**
Ceramics HA [8] TCP[9]	polymers synthetic polyesters, such as PGA, PLA and polycaprolactone.e.g. PGA meshes[16]
Natural polymers like hyaluronic acid,[10] Alginate,[11]Agarose,[12] collagen,[13] Albumin[14]Chitosan.[15]	Co polymers of polyethylene oxide, and polypropylene oxide known as "pluronics"[17] PLGA a copolymer of PGA and PLA.PLGA copolymer Foam.[18]PLGA/HA matrices.[19] Polyphosphazenes.[20] Nano calcium sulphate.[21]

HA- hydroxyapatite; TCP- Tricalcium phosphate; PGA- Polyglycolic acid; PLA- Polylactic acid; PLGA- Poly (lactic-co-glycolic acid)

Methods Of invention Of Scaffolds

- strategy of fibres into nonwoven bone and woven structures.[22]
- integration of sacrificial pore forming agents including ice and soluble particles (eg. Sodium chloride and sucrose.[23]
- Self-assembling molecules.(eg. Certains peptides[24] and collage hydroxyapatitie composites)[25]

- Use of solid free form fabrication.[26] (computerised control over the complex internal features of scaffolds, such as pore size, porosity, pore distribution and an artificial vascular system.)

The underlying concepts guiding the development of scaffolds can be predicated on the selected biomaterials and/or on the method of production of the scaffold.[22]

Cells

Significant parameter of cell source to consider when applying tissue engineering strategies to restore lost tissues and functions. Stem cells are immature progenitor cells. These cells capable of self-renewal and multi-lineage differentiation through a process of asymmetric mitosis that leads to two daughter cells, one identical to the stem cell (daughter stem cell) and one capable of differentiation into more mature cells (progenitor cells).[27]

Stem cells may be:

- Totipotent, i.e. early embryonic cells (one to three days from oocyte fertilization), which can give rise to all the embryonic tissues and placenta.
- Pluripotent, i.e. embryonic cells from blastocystis (4-14 days after oocyte fertilization), which can differentiate only into embryonic tissues belonging to the inner cell mass (ectoderm, mesoderm, and endoderm).
- Multipotent**,** i.e. embryonic cells from the 14th day onwards, which can give rise to tissues belonging to only one embryonic germ layer (ectoderm or mesoderm or endoderm).[28] Depending on the development stage of the tissues from which the stem cells are isolated, stem cells can be broadly divided into two categories: Adult stem cells and embryonic stem cells.[29-31]

Embryonic stem cells are derived from embryos that are 2–11 days old called blastocysts. They are totipotent cells. Due to ethical concerns and the risk of tumorogenicity and teratoma formation, its use has been restricted to the research field. Adult stem cells are multipotent stem cells, and depending upon their origin, they can be further classified into hemopoetic stem cells and mesenchymal stem cells. Friedenstein and colleagues first identified mesenchymal stem cells in aspirates of adult bone marrow.[32] Among the adults stem cells, bone marrow–derived stem cells or mesenchymal stem cells (MSCs) are adherent, proliferating,

and capable of multi-lineage differentiation having the capability of differentiating into multiple tissue types, including bone, cartilage, muscle, tendon, etc., and hold great potential for autologous cell-based therapy.[31] Another important characteristic of MSCs for regenerative medicine is their potential allogenic use without immunosuppressive therapy.[33] Within the sphere of periodontal tissue engineering, mesenchymal derived cells have been applied for simultaneous regeneration of the attachment apparatus components.

Signals

Signaling molecules are proteins that may act locally or systemically to affect the growth and function of cells in various manners. The two types of signaling molecules that have received the greatest attention are growth factors and morphogens that act by altering the cell phenotype i.e. by causing the differentiation of stem cells into bone forming cells -a process commonly known as osteoinduction.

These cytokines have pleotropic effects some of which include

• Mitogenic (proliferative);

•Chemotactic (stimulate directed migration of cells); and

• Angiogenic (stimulate new blood vessel formation) effects.[34]

Growth factors act on the external cell membrane receptors of a target cell, provide the signal to local mesenchymal and epithelial cells to migrate, divide, and increase matrix synthesis. The growth factor that has received the most attention in hard and soft tissue wound healing.

Platelet-derived growth factor

PDGF was one of the first growth factors studied for its effect on wound healing because it is a potent mitogenic and chemotactic factor for mesenchymal cells in cell culture. Platelet-derived growth factor (PDGF) is natural “hormone” of the wound healing. PDGF has been depicted as being the most thoroughly described growth factor associated with the periodontium. There are different forms of PDGF called isoforms and all of them have been shown to have a PDL fibroblast proliferative activity in vitro.It is naturally formed by the body at sites of bone injury and soft tissue. It was discovered by Lynch and coworkers in the late 1980s.[35] While PDGF secreted from platelets play an important role in initial wound healing, its subsequent secretion from macrophages continues the events of wound healing through up-regulation of other growth factors and cells that ultimately promote fibroblastic and osteoblastic functions.[36]

Insulin like growth factor

Insulin like growth factor (IGF) is a potent chemotactic agent for vascular endothelial cells resulting in increased neovascularization. It also stimulates mitosis of many cells *in vitro* such as fibroblasts, osteocytes, and chondrocytes.[37] Insulin like growth factor-I is found in substantial levels in platelets and is released during clotting along with the other growth factors.

Transforming growth factor family

The two best characterized polypeptides from this group of growth factors are Transforming growth factor family (TGF)-α and TGF-β. TGF-β appears to be a major regulator of cell replication and differentiation. Three forms of TGF-β have been identified namely TGF-β1, TGF-β2, and TGF-β3. TGF-β isoforms have multiple regulatory roles in the synthesis, maintenance and turnover of the extracellular matrix. TGF-β is chemotactic for fibroblasts and cementoblasts, and promotes fibroblast accumulation and fibrosis in the healing process. It can also modulate other growth factors such as PDGF, TGF-α, and EGF and fibroblast growth factor (FGF) possibly by altering their cellular response or by inducing their expression.[38]

Fibroblast growth factor family

Fibroblast growth factors are the members of heparin binding growth factor family. The two most thoroughly characterized forms are: Basic FGF (bFGF) and acidic FGF (aFGF). Both aFGF and bFGF are single chain proteins that are proteolytically derived from different precursor molecules to generate biologically active proteins of 15,000 molecular weight. They promote proliferation and attachment of endothelial cells and PDL cells in wound healing process. FGF-2 is known to attract epithelial cells more effectively than FGF-1.[39]

Hepatocyte growth factor

Hepatocyte growth factor (HGF) is a secreted, heparin sulphate glycosaminoglycan-binding protein. HGF has been shown to have mitogenic effects on osteoblasts; thus, participating in the bone remodeling process.

Bone morphogenetic proteins

Bone morphogenetic proteins (BMPs) are the members of transforming growth factor-β (TGF-β) super family, which play a essential role in cell growth and differentiation. They are a group of related proteins that are known to possess the unique ability to induce cartilage and bone

formation.[40] They trigger cellular effects by way of heterotetrameric serine/ threonine kinase receptors and intracellular signaling proteins known as small "mothers against" decapentaplegic (Smads).[41] BMPs, like PDGF, play a role in the blood vessel formation. They play an important role in the angiogenetic activity by up-regulating the angiogenetic peptides like VEGF, may bind to endothelial cells and stimulate the migration and promote blood vessel formation.[35] The hallmark property of BMP is the differentiation factor. BMP will differentiate an undifferentiated mesenchymal cell into an osteoblast. In contrast, PDGF is a chemotactic and mitogenic factor for osteoblast like precursors.[42]

CLINICAL REQUEST OF TISSUE ENGINEERING FOR PERIODONTAL TISSUEREGENERATION

Guided tissue regeneration

The first ones to haveproposed the use of guided tissue regeneration for periodontalregeneration by Nyman and Karring in the 1982. which patent the evolution of periodontalregeneration technologies using tissue engineering. Theplacement of barrier membranes over the denuded rootsurface and the debrided periodontal defect has shownspace provision, epithelial cell occlusion, and exclusion ofgingival connective tissue from the root surface and selectiverepopulation of periodontal ligament cells.

Protein based approaches[43,44]

The use of growth and differentiation factors evolved tissue engineering to its next level and has been the most popular tissue engineering approach for regeneration of periodontal tissues. Several growth factors have been used including

- Transforming growth factor beta; Bone morphogenetic proteins (super family members);
- Basic fibroblast growth factor;
- Platelet derived growth factor.

Enamel matrix derivative

The rationale for the clinical use of enamel matrix derivative is the inspection that enamel matrix proteins are deposited onto the surfaces of developing tooth roots before cementum formation.[45] Enamel Matrix Protein (EMPs) are commercially available as Emdogain which have been known to effect periodontal regeneration. Recent data from a systematic review indicates that biologically EMPs cause an increase in cell attachment of epithelial cells, gingival fibroblasts, and PDL fibroblasts. They increase the expression of transcription factors that are related to chondroblast and osteoblasts/cementoblast

differentiation. Stimulation in the synthesis of total protein and extracellular matrix molecules has also been documented.[46] Use of Enamel matrix derivative (EMD) and a demineralised freeze dried bone allograft (DFDBA) have been demonstrated to be osteopromotive in nature; thus, resulting in an additional increase in bone formation. [47]

Platelet rich plasma

Since physiologic concentrations of growth factors may not be sufficient to stimulate local bone formation, the use of exogenous growth factors to supplement endogenous biological mediators has been explored. Platelet rich plasma (PRP) is a volume of autologous plasma that contains a platelet concentration above baseline values. The development of PRP from autologous blood by simple, sterile (office based and Food and Drug Administration (FDA) cleared devices) by gradient density centrifugation produces a concentration of platelets withvenhanced growth factors including PDGF, TGF-β, and insulin growth factor-1. It has been reported that PRP preparations may increase the concentrations of platelets up to 338%.[48] PRP works through transmembrane receptors and intra cytoplasmic signaling pathways, as do all other growth factor preparations. PRP stimulates the proliferation of human osteogenic cells and periodontal ligament cells.[49] Because PRP and all growth factor preparations work through normal regulated genes and are not autogenous, they are safe promoters of biologic healing and there is no risk of promoting neoplasia.

Recombinant protein therapeutics

With advances in recombinant technology, the development and commercialization of pure recombinant human growth factor-matrix combination has been developed. Combination products which represent the next generation of tissue engineering therapeutics, have gained increasing attention from clinicians and researchers as a strategy to optimize tissue regeneration. Proteins may now be synthesized, concentrated, purified, and packaged in large sterile quantities under tightly controlled and regulated conditions. Providing growth modulating momatrices is important in order to increase the predictability of regenerative procedures. This allows clinical researchers to develop improved regenerative products combining the physical and chemical characteristics of tissue specific matrices required for specific cell attachment, growth and differentiation, with optimal binding and release profile for these bioactive proteins that actively recruit healing cells to the treatment site and expand their cell

numbers, in order to achieve the greatest regeneration.

To date, only three recombinant growth factor products have been widely used

• rh PDGF-BB (gel).[50]

•rhPDGF-BB (with β tricalcium phosphate).[51]

•rh BMP-2 (with type I collagen sponge).[52]

Recombinant platelet derived growth factor (rh PDGF BB) is more than 98% pure recombinant protein developed using conventional recombinant expression techniques under highly controlled conditions. They are first produced by removing the specific DNA sequences from a human cell and transfecting it into a bacterial plasmid. The bacterial plasmid is then transfected into the host cells capable of large scale growth. These are essentially protein factories that synthesize and secrete many proteins. The rh PDGF BB is then separated using sophisticated analytical protein chemistry techniques, sterile filtered and formulated into dose specified for clinical use.[53]

Use of rh PDGF has been one of the options to regenerate the periodontium and has received FDA clearance for use.

The mitogenic responsiveness of periodontal cells to local application of PDGF-BB was confirmed in a dog model by Wang and Castelli. Its levels were raised in cases of periodontitis, but not in diabetic cases; thus, suggesting that PDGF-BB driven repair process is suppressed under diabetic conditions.[54]

The concept of the use of recombinant protein therapeuticsdelivered in an allograft matrix has provided significant clinical results. The efficacy of GEM 21S (growth enhanced matrix β TCP+PDGF), biomimmetic therapeutics were recently reported by Nevins and co-workers.[44]

Role of rhBMP-2 in periodontal regeneration

The identification and development of recombinant human bone morphogenetic protein-2 (rhBMP-2) has lead to the commercial availability for the first time of an osteoinductive autograft replacement (INFUSE® Bone Graft). rhBMP-2 is a homodimeric protein consisting of two BMP-2 protein subunits. Tissue engineering using PRP or recombinant protein therapeutics is a clinical reality in periodontal, cranio maxillofacial, and orthopedics indications. Dental surgeons at long last have access to pure recombinant tissue growth factors, allowing us to progress from previously passive therapies to new active treatments, thereby enhancing the opportunity for regeneration

of bone and other tissues and providing more predictable, faster, less invasive, less traumatic, and efficient outcome for the patient.[51]

Cell based approaches

Cell transplantation using autologous cells to play a central clinical role in the future. Dental cell seeding attempts have attempted to regenerate the periodontal tissues since1990s. Attempts have been made to create the target tissue in the laboratory by culturing and proliferating mesenchymal cells together with scaffolds, before transplanting them into the body.Typical cell harvesting methods using enzymatic dispersion might destroy critical cell surface proteins such as ion channels, while growth factor receptors and cell to cell junctions remain intact. Okano *et al*. developed temperature responsive culture dishes (commercially available under the name of UpCellTM, CellSeed Inc., Tokyo, Japan) by grafting a polymer poly N isopropylacylamide (PIPAAm) onto tissue culture graded polystyrene dishes by irradiation with an electron beam. Cells generally adhere to hydrophobic surfaces, but not to hydrophillic surfaces. At temparatures lower than 32°C, PIPAAm is fully hydrated. This dish allowed intact cells with preserved extracellular matrix proteins and normal cell functions to be harvested with just low temperature treatment.[42] This has evolved into a novel strategy called "Cell sheet engineering" which produces tissues without a specific scaffold. Transplanted cell sheets can be grafted to the recipient tissues without suturing.

Gene delivery based approaches

Numerous tissue regeneration studies have investigated various gene delivery techniques. These techniques involve a gene encoding a therapeutic protein being introduced into the cells which can then express the target protein. This technique avoids the problems associated with the protein delivery method by maintaining constant protein levels at the site of the defect.[2]

Comparative evaluation

Comparison of different agents and techniques used to treat infrabony and furcation defects have been tabulated as in Tables 3-7 and a brief conclusion has been drawn on them:

• Clinical outcome obtained in the EMD patients are similar to those with the use of bio-resorbable membranes. The advantage of using EMD is that they are technically more simple with less risk of exposure, less invasive and resulting in lesser recession after surgery. However, the

adjunctive use of EMD with GTR doesn't seem to enhance the outcome of GTR.

•PRP which provides similar results has the advantage of having hemostatic activity, giving a user friendly environment and acts as a stabilizing agent, immobilizing the blood clot and bone graft from the area.

•The clinical results as well as histologically evaluated periodontal regeneration obtained using rh PDGF and rh BMP produce much superior, but patient centered outcomes, including adverse effects, cost effectiveness, and risk benefit have been evaluated in a very limited number of studies.

•Other bioactive agents are also being experimentally tested to treat periodontal defects including OP-1, transforming growth factor β, b FGF, IGF-1, cementum derived growth factor, vascular endothelial growth factor and many more. More clinical studies need to prove their effectiveness in treating periodontal defects.

Future perceptive

Challenges ahead

1. Structural and functional complexity of the periodontium The fact that more than one tissue must be reconstructed, namely alveolar bone, periodontal ligament, root cementum, and gingiva, makes it much more difficult to find both the right combination and the doses of growth factors.

2. To overcome the rapid clearance of growth factors, a carrier system must be found that stores and releases the growth factors over a longer period of time so that their resident time is prolonged. Although many carrier systems have been tested, none of them appears to be ideal.

3.While high developmental and therapeutic costs appear justified for severe skeletal conditions such as non-unions, open fractures, spinal fusion, and large bone defects, for example in the mandible, the same cannot necessarily be said for relatively small and non-life-threatening periodontal defects where preventive and maintenance measures are still mandatory.

CONCLUSION

Tissue engineering has enlarged our vision and thus made the fascination of being able to achieve regeneration of periodontal complex in its entirety a reality. Though the task has been arduous, but the promise still remain. The study of scaffold materials for use in tissue engineering should lead to improved predictability of this new technology based on cell and molecular biology. In the future it will become increasingly important to consider the concepts of scaffolds that are not only space making and exclusionary, but also

biocompatible and able to elicit appropriate gene expression by the cells for which it is providing the carrier capacity. Understanding the complex design features necessary for successful tissue engineering will help this technique to become an accepted biomedical procedure.

REFERENCES:

1. Illueca FM, Vera PB, Cabanilles PG, Fernanades VF, Loscos FJ.Periodontal regeneration in clinical practice. Med Oral Patol OralCir Bucal 2006;11:382-92.
2. Nakahara T. A review of new developments in tissue engineering therapy for periodontitis. Dent Clin North Am 2006;50:265-6.
3. Abukawa H, Papadaki M, Abulikemu M, Leaf J, Vacanti JP, Kaban LB, et al. The engineering of craniofacial tissues in the laboratory: A review of biomaterials for scaffolds and implant coatings. Dent Clin North Am 2006;50:205-16.
4. Lynch SE. Introduction. In: Lynch SE, Marx RE, Nevins M, Lynch LA, editors. tissue engineering: Applications in Oral and Maxillofacial Surgery and Periodontics. 2nd ed. Chicago: Quintessence Publishing; 2006. p. 11-5.
5. Mohammadi M, Shokrgozar MA, Mofid R. Culture of human gingival fibroblasts on a biodegradable scaffold and evaluation of its effect on attached gingiva: A randomised, controlled pilot study. J Periodontol 2007;78:1897-903.
6. Kao RT, Murakami S, Beirne OR. The use of biological mediators and tissue engineering in dentistry. Periodontol 2000 2009;50:127-53.
7. Spector M. Basic principles of scaffolds in tissue engineering. In: Lynch SE, Marx RE, Nevins M, Lynch LA, editors. Tissue engineering: Applications in Oral and Maxillofacial Surgery and Periodontics. 2nd ed. Chicago: Quintessence Publishing; 2006. p. 26-32.
8. Um YJ, Jung UW, Chae GJ, Kim CS, Lee YK, Cho KS, et al. The effects of hydroxyapatite/calcium phosphate glass scaffold and its surface modification with bovine serum albumin on 1-wall intrabony defects of beagle dogs: A preliminary study. Biomed Mater 2008;3:44113.
9. Stavropoulos A, Windisch P, Szendroi-Kiss D, Peter R, Gera I, Sculean A. Clinical and histologic evaluation of granular Beta-tricalcium phosphate for the treatment of human intrabony periodontal defects: A report on five cases. J Periodontol 2010;81:325-34.
10. Ballini A, Cantore S, Capodiferro S, Grassi FR. Esterified Hyaluronic Acid and Autologous Bone in the Surgical Correction of the Infra-Bone Defects. Int J Med Sci 2009;6:65-71.
11. Lin HR, Yeh YJ. Porous alginate/hydroxyapatite composite scaffolds for bone tissue engineering: Preparation, characterization, and in vitro studies. J Biomed Mater Res B Appl Biomater 2004;71:52-65.
12. Tabata M, Shimoda T, Sugihara K, Ogomi D, Ohgushi H, Akashi M. Apatite formed on/in agarose gel as a bone-grafting material in the

treatment of periodontal infrabony defect. J Biomed Mater Res B Appl Biomater 2005;75:378-86.
13. d'Aquino R, De Rosa A, Lanza V, Tirino V, Laino L, Graziano A, et al. Human mandible bone defect repair by the grafting of dental pulp stem/progenitor cells and collagen sponge biocomplexes. Eur Cell Mater 2009;12:75-83.
14. Chan WD, Perinpanayagam H, Goldberg HA, Hunter GK, Dixon SJ, Santos GC Jr, et al. Tissue Engineering Scaffolds for the Regeneration of Craniofacial Bone. J Can Dent Assoc 2009;75:373-7.
15. Lee YM, Park YJ, Lee SJ, Ku Y, Han SB, Choi SM, et al. Tissue engineered bone formation using chitosan/tricalcium phosphate sponges. J Periodontol 2000;71:410-7.
16. Gunatillake PA, Adhikari R. Biodegradable synthetic polymers for tissue engineering. Eur Cell Mater 2003;5:1-16.
17. Xu XL, Lou J, Tang T, Ng KW, Zhang J, Yu C, et al. Evaluation of different scaffolds for for BMP-2 genetic orthopaedic tissue engineering. J Biomed Mater Res B Appl Biomater 2005;75:289-303.
18. Abukawa H, Terai H, Hannouche D, Vacanti JP, Kaban LB, Troulis MJ. Reconstruction of the mandible condyle by tissue engineering. J Oral Maxillofac Surg 2003;61:94-100.
19. Kim S, Kim SS, Lee SH, Eun Ahn S, Gwak SJ, Song JH, et al. In vivo bone formation from human embryonic stem cell-derived osteogenic cells in poly(d, l-lactic-co-glycolic acid)/ hydroxyapatite composite scaffolds. Biomaterials 2008;29:1043-3.
20. Laurencin CT, Ambrosio AM, Sahota JS. Novel polyphosphazene-hydroxyapatite composites as biomaterials. IEEE Eng Med Biol Mag 2003;22:18-26.
21. Mohan, Kathiravan. Nano calcium sulfate scaffolds for periodontal tissue engineering: Cell-material interaction in vitro studies. State University Of New York At Buffalo 2008: 1456888.
22. Langer R, Vacanti JP. Tissue engineering. Science 1993;260:920-6.
23. Yannas IV, Lee E, Orgill DP, Skrabut EM, Murphy GF. Synthesis and characterisation of a model extracellular matrix that induces partial regeneration of adult mammalian skin. Proc Natl Acad Sci U S A 1989;86:933-7.
24. Zhang S. Fabrication of novel biomaterials through molecular self assembly. Nat Biotechnol 2003;21:1171-8.
25. Liao SS, Cui FZ, Zhang W, Feng QL. Hierarchically biomimmetic bone scaffold materials. Nano-HA/collagen/PLA composite. J Biomed Mater Res 2004;69 B:158-65.
26. Sun W, Yan Y, Lin F, Spector M. Biomanufacturing: A US-china national science foundation sponsored workshop. Tissue Eng 2006;12:1169-81.
27. Nadig RR. Stem cell therapy- hype or hope. A review. J Conserv Dent 2009;12:131-8.
28. Krampera M, Franchini M, Pizzolo G, Aprili G. Mesenchymal stem cells: From biology to clinical use. Blood Transfus 2007;5:120-9.
29. Shamblott MJ, Axelman J, Wang S, Bugg EM, Littlefield JW, Donovan PJ, et al. Derivation of pluripotent stem cells from cultured human primordial germ cells. Proc Natl Acad Sci U S A 1998;95:13726-31.

30. Thomson JA, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS, et al. Embryonic stem cell lines derived from human blastocysts. Science 1998;282:1145-7.
31. Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, et al. Multilineage potential of adult human mesenchymal stem cells. Science 1999;284:143-7.
32. Friedenstein AJ. Precursor cells of mechanocytes. Int Rev Cytol 1976;47:327-59
33. Aggarwal S, Pittenger MF. Human mesenchymal stem cells modulate allogeneic immune cell responses. Blood 2005;105:1815-2.
34. Lynch SE, Lynch LA, Nevins M. Use of rhPDGF to improve bone and periodontal regeneration. In: Lynch SE, Marx RE, Nevins M, Lynch LA, editors. Tissue engineering: Applications in Oral and Maxillofacial Surgery and Periodontics. 2nd ed. Chicago: Quintessence Publishing; 2006. p. 87-102.
35. Lynch SE, de Castilla GR, Williams RC, Kiritsy CP, Howell TH, Reddy MS, et al. The effects of short term application of a combination of platelet-derived and insulin growth factors on periodontal wound healing. J Periodontol 1991;62:458-67.
36. Pryor ME, Polimeni G, Koo KT, Hartman MJ, Gross H, April M, et al. Analysis of rat calvaria defects implanted with a platelet rich plasma preapartion: Histological and histometric observations. J Clin Periodontol 2005;32:966-72.
37. Bennett NT, Schultz GS. Growth factors and wound healing: Biochemical properties of growth factors and their receptors. Am J Surg 1993;165:728-37.
38. Hollinger J, Buck D, Bruder SP. Biology of bone healing: Its impact on clinical therapy. In: Lynch, Samuel E and Genco, Robert and Marx, Robert, editors. Tissue Engineering: Applications in Maxillofacial Surgery and Periodontics. 1st ed. Chicago: Quintessence Publishing; 1999. p. 17-53
39. Raja S, Byakod G, Pudakalkatti P. Growth factors in periodontal regeneration. Int J Dent Hygiene 2009;7:82-9.
40. Hou LT, Liu CM, Liu BY, Chang PC, Chen MH, Ho MH, et al. Tissue engineering bone formation in novel recombinant human bone morphogenetic protein-2 atellocollagen sponge composite scaffolds. J Periodontol 2007;78:335-43.
41. Heliotis M, Tsiridis E. Suppression of bone morphogenetic protein inhibitors promotes osteogenic differentiation: Therapeutic implications. Arthritis Res Ther 2008;10:115.
42. Okano T, Bae YH, Jacobs H, Kim SW. Thermally on- off swithching polymers for drug permeation and release. J Control Release 1990;11:255-5.
43. Murakami S, Takayama S, Kitamura M, Shimabukuro Y, Yanagi K, Ikezawa K, et al. Recombinant human basic fibroblast growth factor stimulates periodontal regeneration in class II furcation defects created in beagle dogs. J Periodontol Res 2003;38:97-3.
44. Nevins M, Camelo M, Nevins ML, Schenk RK, Lynch SE. Periodontal regeneration in humans using recombinant human platelet derived growth factor BB and allogeneic bone. J Periodontol 2003;74:1282-92.

45. Gestrelius S, Andersson C, Lidström D, Hammarström L, Somerman M. In vitro studies on periodontal ligament cells and enamel matrix derivative. J Clin Periodontol 1997;24:685-92.
46. Bosshardt DD. Biological mediators and periodontal regeneration. A review of enamel matrix proteins at the cellular and molecular levels. J Clin Periodntol 2008;35 Suppl:87-105.
47. Boyan BD, Weesner TC, Lohmann CH, Andreacchio D, Carnes DL, Dean DD. Porcine fetal enamel matrix derivative enhances bone formation induced by demineralized freeze dried bone allograft in vivo. J Periodontol 2000;71:1278-86.
48. Marx RE. Platelet rich plasma(PRP): What is PRP and what is not PRP? Implant Dent 2001;10:225-8.
49. Okuda K, Kawase T, Momose M, Murata M, Saito Y, Suzuki H,et al. Platelet rich plasma contains high levels of plateletderived growth factor and transforming growth factor beta and modulates the proliferation of periodontally related cells in vitro.J Periodontol 2003;74:849-57.
50. Huang JS, Huang SS, Deuel TF. Human platelet derived growth factor. Radioimmunoassay and discovery of a specific plasma binding protein. J Cell Biol 1983;97:383-8.
51. McGuire MK, Scheyer ET. Comparison of recombinant human platelet derived growth-factor bb plus beta tricalcium phosphate and a collagen membrane to subepithelial connective tissue grafting for the treatment of recession defects: A case series. Int J Periodontol Rest Dent 2006;26:127-3.
52. Selvig KA, Sorensen RG, Wozney JM, Wikesjo UM. Bone repair following recombinant human bone morphogenic protein-2 stimulated periodontal regeneration. J Periodontol 2002;73:1020-9.
53. Marx RE. Application of tissue engineering principles to clinical practice. In: Lynch SE, Marx RE, Nevins M, Lynch LA, editors. tissue engineering: Applications in Oral and Maxillofacial Surgery and Periodontics. 2nd ed. Chicago: Quintessence Publishing; 2006.p. 47-63.
54. Giannobile WV. Periodontal tissue engineering by growth factors. Bone 1996;19(1 Suppl):23S-37S.