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Tissue Engineering In Periodontal Regeneration: A Review

[1] Pooja Palwankar
[2] Kapil Arora
[3] Minakshi Rana

[1] HOD, Dept. of Periodontology
[2] HOD, Department of Public Health Dentistry
[3] PG Student, Dept. of Periodontology
Manav Rachna Dental College

Address For Correspondence:
Dr. Minakshi Rana, Post Graduate Student,
Department of Periodontology
Manav Rachna Dental College, Faridabad
Phone No.: 09871941609
E mail ID: minakshirana28@gmail.com
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Abstract
Tissue engineering is a highly promising field of reconstructive science that draws on recent advances in medicine, surgery, molecular and cellular biology. The objective of tissue engineering is to facilitate healing processes by using synthetic polymers, which are intended to allow growth, specialization of cells and then get bioabsorbed and replaced by natural physiologic tissues over time. In periodontology, the concept of tissue engineering had its beginning with guided tissue regeneration, a mechanical approach utilizing barrier membranes to obtain regeneration in defects. With the availability of partially purified protein mixture from developing teeth and growth factors from recombinant technology, a new era of tissue engineering has emerged whereby biologic mediators can be used for periodontal regeneration. It is true that new approach in form of tissue engineering has reduced complexity of achieving periodontal regeneration. Therefore, we should have confidence in the future for investigations on tissue engineering techniques, which will, without doubt, bring new and highly useful knowledge to the clinical practice.

Key Words
Tissue Engineering, Synthetic Polymers, Periodontal Regeneration, Growth Factors

Introduction:

Periodontitis, inflammation of periodontal apparatus; clinically manifests as destruction of tooth supporting structures. The ultimate goal of periodontal therapy is to completely restore tooth supporting structures or the periodontal attachment including cementum, periodontal ligament and alveolar bone lost during periodontal disease. In past few decades, many attempts have been made to unravel the "Magic molecules" that could result in new clinical and histological attachment; hence resulting in healthy functional tissue.

During healing after the periodontal therapy, the tissue can undergo either repair or regeneration, Repair refers to healing of tissue without maintaining its original morphology and function, and it is considered as non-functional scarring. Regeneration attributes to complete recovery of periodontal tissues in both structure and function,that is the formation of alveolar bone, a new connective tissue attachment through collagen fibres functionally oriented on newly formed cementum.[1] Regeneration of periodontal tissue is a complex phenomenon requiring interplay between various processes in timely manner. 'Tissue engineering' term was coined in 1987 at national science foundation bioengineering meeting in Washington D.C. It was coined to denote lab construction of a device containing viable cells and biologic mediators in synthetic or biologic matrix that could be implanted in patients to facilitate regeneration of particular tissues.[2]

Definitions of tissue engineering:

Vacanti and Langer (1987), defined tissue engineering as "A combination of principles and methods of life services with that of engineering, to develop materials and methods to repair damage or diseased tissues, and to create a entire tissue replacements.[3]

Shalak and Fox (1988), defined it as application of principles and methods of engineering and life sciences, to obtain a fundamental understanding of structural and functional relationships in novel and pathological mammalian tissues, and development of biological substitutes to restore, maintain or improve tissue formation.[4]

The goal of tissue engineering is to promote healing and to cause true regeneration of tissue structure and function more quickly, less invasively and more quantitatively. The purpose behind writing the review is to integrate the tissue regenerative methods available and to implement them in routine practice in an evidence based manner.

Passive to Active: A Tissue Engineering Approach

The complexity of periodontal healing is attributed to open environment for healing which is permanently contaminated and under significant bacterial load. Adding to this is the continuous occlusal load on tooth in transverse and axial axis, which affect stability of wound healing.[5] Various approaches viz bone grafts and barrier membranes have been used, but none has been proved efficient in achieving complete functional connection. The natural wound healing process usually results in tissue scarring or repair; in order to manipulate natural healing process tissue engineering enters in to the scenario. The tissue engineering approach to bone and periodontal regeneration combines 3 key elements:

1. Conductive scaffold/extracellular matrix
2. Signaling molecules
3. Stem cells / progenitor cells[6]

The combined effort of theses key elements results in tissue regeneration and the three components involved in tissue engineering are: **(Figure-1)**

A) Scaffold

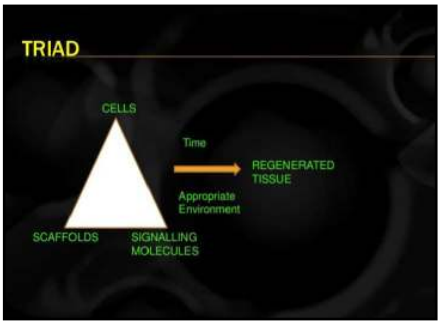

Fig - 1 : Triad Of Tissue Engineering

B) Cells
C) Signaling molecules

A. Scaffolds:

Scaffold provides a 3D substratum on to which cells can proliferate and migrate, produce a matrix and form a functional tissue with desired shape.[7] Synthetic and natural calcium phosphates and myriad synthetic (Polylactic acid and Polyglycolic acid) and natural polymers (collagen and fibrin) are included as matrices for engineering bone and soft tissue. A scaffold should have microstructure and chemical composition required for normal cell growth and function.[8]

Function of matrices/scaffold in regenerative process includes.[7]

a. Structural reinforcement
b. Barrier to in-growth of surrounding tissue
c. Scaffold for migration and proliferation of cells
d. Insoluble regulator of cell function through its interaction with certain integrins

The desirable characteristics of these materials are biocompatibility (i.e., not to provoke any unwanted tissue response to the scaffold, and at the same time to possess the right surface chemistry to promote cell attachment and function) and biodegradability (i.e., degradable into nontoxic products, leaving the desired living tissue).[9] Along with these properties high strength matrix material with modulus of elasticity of tissue comparable with that of surrounding tissue is preferred to be used as scaffolds. Liao et al (2010)[10] compared ß - tricalcium phosphate/ chitosan composite scaffold with pure chitosan scaffold. Composite scaffold shows higher proliferation of human periodontal stem cells and upregulated the gene expression of sialoprotein and cementum attachment protein. In-vivo high performance liquid chromatography in the composite scaffold not only proliferated but, but also recruited vascular tissue ingrowth; thus suggesting benefit of using these composite scaffold.Various materials are used as scaffolds. **Table - 1**

Table - 1 : Materials Used As Scaffold

Natural	Synthetic
1. Ceramic 2. Hydroxyapatite 3. Tricalcium Phosphate 4. Polymers – Hyaluronic Acid, Alginate, Agarose, Chitosan, Collagen And Albumin	1. Polymers – Polyglycolic, Polylactic, 2. Polycaprolactone 3. Co-polymers Of Polyethylene Oxide And Polypropylene Oxide 4. Polyphosphagenes 5. Nano Calcium Sulphate

Metals-Over the past century, biocompatible materials such as metals, ceramics, and polymers have been used extensively for surgical implantations. Metals and ceramics have contributed to major advances in medicine, particularly in orthopedic tissue replacements. Typical implant metals are stainless steels, cobalt based alloys, and titanium-based alloys, and typical ceramics are alumina, zirconia, calcium phosphate, and bioglass. Hip endoprosthesis is a typical device made from these materials which has remarkably improved the quality of life of many people after hip replacement surgery. However, metals and ceramics have two major disadvantages for tissue engineering applications. First, they are not biodegradable (except biodegradable bioceramics such as a-tricalcium phosphate, b-tricalcium phosphate), and second, their processability is very limited. For these reasons, polymeric materials have received an increasing amount of attention from the scientific and medical communities.[9]

Polymers-Natural polymers, such as collagens, glycosaminoglycan, starch, chitin, and chitosan, have been used to repair nerves, skin, cartilage, and bone. While naturally occurring biomaterials may most closely simulate the native cellular milieu, large batch-to-batch variations upon isolation from biological tissues is the main limitation for their wide applications. Poor mechanical performances is also a drawback for transplantation scaffolds made from natural polymers, such as collagen and chitin, which cannot be easily melted with heat but require a special solvent. Many synthetic resorbable polymers, such as poly (a-hydroxy esters), polyanhydrides, polyorthoesters, and polyphosphazens, have been developed to overcome the aforementioned problems associated with natural polymers. Most synthetic polymers are degraded via chemical hydrolysis and insensitive to enzymatic processes so that their degradation does not vary from patient to patient. An important class of synthetic resorbable polymers includes poly (a-hydroxy esters) and copolyesters of the lactic acid and glycolic acid. In the United States, polyglycolic acid, or polyglycolide (PGA), polylactic acid, or polylactide (PLA), polydioxanone, and copolymers; are the only synthetic, degradable polymers with an U.S. Food and Drug Administration (FDA) approval. They have been in use for over 20 years in surgical sutures, and have a long and favorable clinical record. By far, the family of PLA is the most commonly used synthetic biomaterial. A wide range of physical properties and degradation times can be achieved by varying the monomer ratios in lactide/glycolide copolymers poly- L-lactide (PLLA) and PGA exhibit a high degree of crystallinity and degrade relatively slowly, while copolymers of PLLA and PGA (i.e., PLGA) are amorphous and degrade rapidly.[9]

B. Cells:

It is an important parameter to consider when applying tissue engineering strategies to restore lost tissue and function. Stem cells are cells having regenerative potential, they are immature progenitor cells capable of cell renewal and multi-lineage differentiation through a process of asymmetric mitosis leading to production of progenitor stem cell and daughter cell.[11] On basis of regenerative potential, stem cells can be -

i. Totipotent cells – differentiate in to embryonic and extra-embryonic cells, able to construct complete viable organism. E.g blastocyst
ii. Pluiripotent cells – cells derived from germ layers, differentiate in to any type of cells but not complete organism.
iii. Multipotent cells – give rise to limited range of cells in a tissue. E.g hematopoietic stem cells
iv. Oligopotent – differentiate in to few type of cells. E.g lymphoid and myeloid precursor cells.
v. Unipotent – produce only one type of cell i.e have self renewal property e.g muscle stem cell[12]

On the basis of source of origin stem cells can further be classified in to:

a. Autologous cells
b. Allogenic cells
c. Xenogenic cells.

Table - 2 : Action Of Various Growth Factors During Wound Healing

Growth Factor	Fibroblast Proliferation	Osteoblast Proliferation	Mesenchymal Cell Differentiation	Vascularization	Extracellular Matrix Synthesis
Egfs	+ +	-	+ +	+	-
Fgfs	+ +	+ +	-	+ +	-
Pdgfs	+ +	+ +	-	+ (Indirect Effect)	-
Igfs	+	+ +	-	-	+ +
Tgf-beta	+ Or -	+ Or -	-	+ (Indirect Effect)	+ +
Bmps	-	±	+ +	+ + (Indirect Effect)	±

Autologous stem cells are most safe to be used due to no antigenic property.[6] Autologous stem cells can be embryonic stem cells and adult stem cells. Embryonic stem cells involve cells derived from inner cell mass, placenta and umbilical cord. Adult stem cells are derived from bone marrow, Periodontal ligament (PDL) and dental pulp.[13] Seo et al (2004)[14] have identified mesenchymal stem cells for the first time derived from adult PDL which is known as Periodontal ligament stem cells (PDLSCs).

Various modes used for supplying cells to the defected site involve:

I. Cell seeding
II. Cell suspension

Cell seeding involves incorporation in to implantable matrices, ensuring its localization at treatment site.A bioreactor system that supports long-term Stem Cells growth and three-dimensional (3-D) tissue formation is utilized for cell seeding technique. A 3-D perfusion bioreactor system was designed using non-woven polyethylene terepthalate (PET) fibrous matrices as scaffolds. Its main features are its modular design and integrated seeding operation. Cell suspension technique involves culturing stem cells in culture media in-vitro and then to inject cells in to sealed compartment containing defect.[15]

C. Signaling molecules:

Signaling molecules are proteins that may act systemically and locally to effect growth and function of cells in various manners.[7] Systemically acting signaling molecules includes hormones like growth hormone, parathyroid hormone, leutinizing hormone etc. locally active signaling molecules includes platelet derived growth factor (PDGF), transforming growth factor – alpha (TGF- alpha), transforming growth factor- beta (TGF- ß), bone morphogenetic protein (BMP's), parathyroid hormone recombinant protein (PTHrP). These molecules exhibit pleotropic effect some of which include mitogenic, chemotactic and angiogenic. Growth factors acts on external cell membrane receptors of a target cell, provide signal to mesenchymal and epithelial stem cells to migrate, divide and increase matrix synthesis.[7] Growth factors plays major role during wound healing[16]. **(Table-2)**

Clinical application of tissue engineering for periodontal regeneration:

In periodontology, the concept of tissue engineering had its beginnings with guided tissue regeneration, a mechanical approach utilizing nonresorbable and resorbable membranes to obtain regeneration in defects. In dental implantology, guided bone regeneration membranes are used for bone augmentation of proposed implant placement sites. With the availability of partially purified protein mixture from developing teeth and growth factors from recombinant technology, a new era of tissue engineering has emerged whereby biologic mediators can be used for periodontal regeneration[16]

Fig - 2 : Growth Factor & Bone Matrix At Periodontal Healing Site

Basic Approaches To Tissue Engineering In Periodontics: [6]

1. Protein based approach
2. Cell based approach
3. Gene therapy approach

Protein Based Approach- It involves use of growth factors and differentiation factors for causing regeneration. **(Fig-2)**. Various growth factors found to be involved during wound healing are – PDGF, TGF- alpha, MDGF, MDAF, bFGF, BMP. **(Table - 3)**

Platelet Derived Growth Factor (PDGF) - Lynch and co-workers discovered PDGF in 1980 which is a natural wound healing hormone produced by body at site of soft tissue and bone injury.[17] Its initial secretion at wound healing site stimulates mitogenesis of marrow stem cells and its subsequent synthesis from macrophages continues event of wound healing by up regulation of various other growth factors that ultimately promotes fibroblastic and osteoblastic function.[7]

Insulin derived growth factor (IGF) - It is a potent chemotactic agent for vascular endothelial cells resulting in increased neo-vascularisation. It also stimulates mitosis of cells e.g. fibroblast, osteocyte and chondrocytes. Insulin like growth factor-1 is found in substantial levels in

Table - 3 : Sources Of Growth Factors

Growth Factor	Alternative Names	Source
Platelet Derived Growth Factor	Fibroblast Derived Growth Factor Glioma Derived Growth Factor	Degranulating Platelets Endothelial Cells, Smooth Muscle, Mcrophages,Fibroblast, Osteoblasts, Osteoclasts, Mesenchymal Stem Cells
Insulin Like Growth Factor	Erythropoetic Factor Growth Promoting Activity For Vascular Endothelial Cells	Macrophages, Osteoblast, Plasma Stored In Bone, Epithelial Cells Endothelial Cells, Fibroblasts, Smooth Muscle Cells
Tgf-alpha	Milk Derived Growth Factor Transformed Growth Factor	Macrophages,Osteoblasts, Platelets
Tgf-beta	Epithelial Cell-specific Growth Inhibitor Tumor Inducing Factor-1	Platelet Alpha Granules, Macrophages, Osteoblast, Activated T-lymphocytes, Immature Chondrocytes
Fibroblast Growth Factor Afgf Bfgf	Heparin Binding Growth Factor –alpha Adipocyte Growth Factor Bone Derived Growth Factor	Macrophages, Osteoblasts, Endothelial Cells, Immature And Mature Chondrocytes
Hepatocyte Growth Factor		Mesenchymal Stem Cells

platelets and is released during clotting along with other growth factors.[7]

Han and Amar (2003)[18] demonstrated that IGF-1 substantially enhanced cell survival of periodontal ligament fibroblast as compared to gingival fibroblast by up-regulation of anti-apoptotic molecules and down regulation of pro-apoptotic molecules.

Transforming growth factor (TGF) - The two best characterized polypeptide from this group of growth factor are transforming growth factor- alpha (TGF-alpha) and transforming growth factor –beta (TGF-ß). TGF- ß appears to be a major regulator of cell replication and differentiation. Three forms of TGF- ß have been identified namely TGF- ß1, TGF- ß2, and TGF- ß3. TGF- ß have multiple regulatory roles in synthesis, maintenance and turnover of extracellular matrix. TGF- ß is chemotactic for fibroblast and cementoblast and promotes fibroblast accumulation and fibrosis in healing process. It can also modulate other growth factor such as PDGF, TGF-alpha, epithelial growth factor (EGF), fibroblast growth factor (FGF) possibly by altering their cellular response or by inducing their expression.[7]

Oates et al (1993)[19] compared the chemotactic potential of TGF- ß with interleukin-1 and PDGF in fibroblast cells derived from periodontal ligament explants. TGF- ß was relatively a weak mitogen for periodontal cells compared to PDGF, suggesting TGF- ß may indirectly stimulate DNA synthesis.

Fibroblast growth factor (FGF) - FGF is a member of heparin growth factor family. The two most characterized forms are: aFGF(acidic) and bFGF (basic). These are single chaon protein molecules that promote proliferation and attachment of endothelial cells and PDL cells in wound healing process.[20]

Kitamara et al (2008)[21], conducted a randomized clinical trial to evaluate therapeutic response of varying levels of bFGF. They demonstrated a significant increase in alveolar bone height on using 0.3% FGF.

Takayama et al (2001)[22] examined efficiency of topical application of FGF-2 in surgically created bone defects in non-human primates and concluded that topical application FGF-2 enhance considerable periodontal regeneration.

Bone morphogenetic protein (BMP) - Lynch (1993)[6] stated that specific bone forming substance activate non-specific mesenchymal tissue. BMP's are considered as the substance regulating bone formation. The bone morphogenetic protein genes were cloned for the first time by Wozney in 1988. BMP's are members of transforming growth factor superfamily. They are categorized in to 13 categories numbered from 1-13; BMP-1 is a protease that activates other BMP's. BMP-7, 8, 9 is an osteogenic protein for alveolar bone. Wang synthesize oligonucleotide probe for isolating and screening bone genomic libraries that encode for human BMP's. BMP's are regulated by homeobox genes, which provide spatial and temporal information to cells during differentiation and proliferation. Many studies [23],[24],[25] have proved therapeutic effect of using BMP's in periodontal regeneration and also reported beneficial effect.

Platelet rich plasma / Platelet rich fibrin - Regenerative potential of platelets was introduced in 1974, and Ross et al. were amongst the pioneers who first described a growth factor from platelets. Growth factors released after activation from the platelets trapped within fibrin matrix, and have been shown to stimulate the mitogenic response in the periosteum for bone repair during normal wound healing.[26] Last two decades has seen the better understanding of physiologic properties of platelets in wound healing that led to increased therapeutic applications in the various forms with varying results. However, controversies owing to the complexity of the production protocols for autologous fibrin adhesives or risk of cross infection for commercial adhesives, along with legal restrictions on blood handling with concentrated platelet rich plasma (cPRP), a new family of platelet concentrate, an autologous cicatricial matrix, platelet rich fibrin (PRF) appeared in France.[27] Choukroun's platelet rich fibrin (PRF) is a fibrin matrix in which platelet cytokines and cells are trapped which are released after a certain time and that can serve as a resorbable membrane. More recently, Gassling et al. have shown that PRF is a suitable scaffold for breeding human periosteal cells in vitro, which may be suitable for bone tissue engineering applications.[28]

Cell Based Approach -
Cell transplantation using autologous cell seems to be a central futuristic approach in regenerative procedures. Attempts have been made to create target tissue in the laboratory by culturing and proliferating mesenchymal cells together with scaffolds, before transplanting them in to body. In cell based therapeutic approaches, stem cells and/or progenitor cells are manipulated in vitro and administered to patients as living and dynamic biological agents.[12] Seo et al (2004)[14] have identified mesenchymal stem cells for the first time derived from adult PDL which is known as PDL stem cells (PDLSCs). PDLSCs represent a novel population of multipotent stem cells, as shown by their capacity to develop into cementoblast-like cells and adipocytes in vitro and cementum/PDL-like tissue in vivo. PDLSCs also demonstrated the capacity to form collagen fibers, similar to Sharpey's fibers, connecting to the cementum-like tissue, suggesting the potential to regenerate PDL attachment.[16]

Gene Therapy Approach -
Genes are called as code of life. Kinane (2005), introduced term gene therapy which includes the process of genetic modification of cells for therapeutic purposes. It was initially in 1999, that genetic modification was used for treating SCID (severe combined immunodeficiency syndrome), which had given successful results, following its result it has proven beneficial effects in treatment of malignant melanoma (2006), inherited retinal disease (2007), and trichromatic vision in squirrel monkey (2009). Implication of gene therapy in periodontics includes both in-vivo and ex-vivo approach. In-vivo approach involves using systemic infusion, tissue injection and biolistic gene gun. In periodontics, gene therapy has been used for improvement in synthesis of platelet derived growth factor and bone morphogenetic protein. Gene therapy enhanced tissue engineering has led to evolution of periodontal vaccination, treatment of biofilm antibiotic resistance, electroporation, antimicrobial gene therapy and designer drug therapy.[29]

I. **Gene therapeutics** - periodontal vaccination: specific salivary immunoglobulins IgG, IgA antibodies and serum IgG antibodies were produced against Porphyromonas gingivalis fimbraie producing activity plasmid DNA inoculation in rats. Scientist have demonstrated the effectiveness of using these genetically engineered antibodies as vaccine for inhibiting P.Gingivalis fimbraie producing gene.

II. **Gene approach to biofilm antibiotic resistance :** Mah et al; 2003[30] identified gene ndvB encoding for glycosyltransferase required for the synthesis of periplasmic glucans in Pseudomonas aeuroginosa RA14 strain and this glucan layer is responsible for its antibiotic resistance. Using genetic approach, mutant of ndvB gene of pseudomonas aeruginosa have been isolated, capable of forming biofilm but lacking property of glucan formation, hence preventing antibiotic resistance.

III. **Electroporation for bone remodeling :** Using an in vivo transfer of LacZ gene (gene encoding for various remodeling molecules) into the periodontium and using plasmid DNA as a vector along with electroporation (electric impulse) for driving the gene into cell, has shown predictable alveolar bone remodeling.

IV. **Antimicrobial gene therapy for controlling disease progression :** host cells when infected in vivo with β defensin-2 (HBD-2) gene via retroviral vector; there was a potent antimicrobial activity which enhanced host antimicrobial defenses, controlling disease progression.

V. **Designer drug therapy :** If genes necessary for normal development are known, then

VI. **"designer drug therapies"** aimed at one area of the gene or the other can be developed. These designer drugs will be safer than today's medicines because they would only affect the defect in a gene clearly identified through genetic research.

Certain technical difficulties are found associated with application of gene therapy[29]:

- Difficulty in delivering the gene
- Short lived nature of gene therapy
- Activation of immune response
- Chances of inducing a tumor
- Safety of vector
- Difficulty in treating multigene transfer
- Expensive therapy

Challenges ahead in tissue engineering of periodontium:

- Structural and functional complexity of Periodontium – Periodontium involves both soft and hard tissue, arranged in structural harmony with one another. Engineering of complete periodontium poses problem as it requires presence of different cell lineages i.e cementoblast, fibroblast and osteoblast. For the periodontal ligament fibres to get entangled in to surrounding hard tissue components all three cell lineages are required simultaneously at periodontal healing sites, along with their functional integrity.
- Appropriate vector for long term availability of growth factors.
- Cost appear justified for severe life threatening conditions, same not true for non-life threatening periodontal defects where preventive and maintenance measures are still mandatory.

Conclusion:

We need to look beyond before we can achieve dream of complete periodontal regeneration. Tissue engineering has enlarged our vision and made fascination of achieving periodontal regeneration a reality. It has emerged from its stage of infancy of mere theoretical and hypothetical quotations to scientific researches, which reveals potential hopes. There are still lots of researches and details of mechanisms needed to be understood to incorporate it as a new treatment modality.

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