

“Tissue Engineering in Periodontics : A Review”

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ABSTRACT:

Tissue engineering is the emerging field of science, developing techniques for fabrication of new tissues for replacement based on principles of cell and developmental biology and biomaterials. Tissue engineering is a relatively new field of regenerative medicine which utilizes mechanical, cellular, or biologic mediators to facilitate reconstruction/regeneration of a particular tissue.

In periodontology, the concept of tissue engineering had its beginnings with guided tissue regeneration, a mechanical approach utilizing nonresorbable 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. The article reviews various aspects of tissue engineering for periodontal tissue regeneration which may act as promising tool for restoration of damaged periodontal tissues.

Key Words: Growth factors, Periodontal regeneration, GTR, Tissue Engineering.

INTRODUCTION

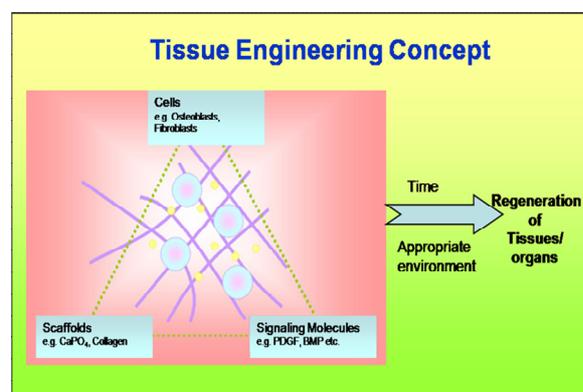
Tissue and organ failure, produced as a result of injury or other type of damage, is a major health problem with treatment option involving transplantation, surgical repair, artificial prostheses, mechanical devices etc.¹

Tissue engineering is emerging as a significant potential alternative or complementary solution, whereby tissues or organ failure is addressed by implanting natural, synthetic or semi synthetic tissues and organ mimics that are fully functional from the start, or that grows into the required functionality.²

Tissue engineering is the branch of biology where tissues are produced in culture by cells seeded (grown) in various porous absorbable matrices by using biological principles.¹

PRINCIPLES OF TISSUE ENGINEERING

Using tissue engineering, the wound healing process is manipulated so that tissue regeneration occurs. This manipulation usually involves one or more of the following three key elements: the signaling molecules; scaffold or supporting matrices; and cells.³(Figure 1)



- Langer M et al, 1993

Fig.1 key Elements for Tissue Engineering

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Cells provide the machinery for new tissue growth and differentiation. Growth factors or morphogens modulate the cellular activity and provide stimuli to cells to differentiate and produce matrices toward the developing tissue. New vascular networks promoted by angiogenic signals provide the nutritional base for tissue growth and homeostasis. Finally, scaffolds guide and create a template three-dimensionally to facilitate the above processes critical for tissue regeneration⁴.

Earliest literature on tissue engineering lies in field of Periodontology as GTR & GBR.

Guided tissue regeneration (GTR)

The biological principle of using cell-occlusive barriers was described by Melcher (1976)⁵. GTR consists of placing

barriers of different types to cover the bone and periodontal ligament, thus temporarily separating them from the gingival epithelium. This provides space and favorable niche to guide right type of cells (PDL cells, cementoblasts and osteoblasts) to attach at the root surface, and tries to exclude undesirable cells (epithelial cells) from attaching to root surface.

Guided bone regeneration (GBR)

GBR involves use of membranes to guide bony tissue formation by separating the underlying bone from the overlying connective tissue & by creating a space into which the desirable bone cells can migrate.⁶ This is usually done before implant placement for bone augmentation. (Table 1)

Table 1: **Cell occlusive barriers used for Periodontal Regeneration⁸**

Cell occlusive barriers	Trade name
1. Non resorbable Cellulose, ePTFE (Expanded poly tetra-fluoroethylene)	Milipore filter ^R , Gore-Tex ^R
2. Resorbable Polylactic acid and poly-glycolic acid, Polyglactin-910, poly(L-lactide)	Resolut ^R , Atrisorb ^R , Vicryl-Netz ^R
3. Collagen Bovine tendon type I, Porcine dermis type I + III	Biomed ^R , BioGide ^R , Ossix ^R
4. Plaster of Paris Calcium sulfate	Cap-set ^R , Hap-set ^R

SCAFFOLD OR SUPPORTING MATRICES (Table 2)

It should have following features⁷ :

i) Biomechanical features

1. *Space maintenance*

2. *Barrier or exclusionary features*

ii) Biological functions

1. *Biocompatibility*

2. *Incorporation of cells*

3. *Incorporation of instructive messages*

Table 2: Scaffold materials used for Periodontal Repair and Regeneration⁸

Biomaterial	Trade name
1. Allografts Calcified freeze-dried bone, Decalcified freeze-dried bone	Grafton ^R , Lifenet ^R , Musculoskeletal Trasnplant Foundation ^R
2. Xenografts Bovine mineral matrix, bovine-derived hydroxyapatite (HA)	Bio-Oss ^R , OsteoGraft ^R , Pep-Gen P - 15 ^R
3. Alloplasts Hydroxyapatite (Dense HA, Porous HA, Resorbable HA) Tricalcium phosphate. Calcium phosphate cement Hard tissue replacement polymers Bioactive glass (SiO ₂ , CaO, Na ₂ O, P ₂ O ₅) Coral-derived calcium carbonate	Osteogen ^R , Periograft ^R , ProOsteone ^R Synthograft ^R , α-BSM ^R , Bioplant ^R PerioGlass ^R , BioGran ^R Biocoral ^R
4. Polymer and collagens Collagen Poly(lactide-co-polyglycolide) Methylcellulose Hyaluronic acid ester Chitosan	Helistat ^R , Collacote ^R , Colla-Tec ^R , Gelfoam ^R Hy ^R
5. Enamel matrix derivative	Emdogain ^R

USE OF SIGNALING MOLECULES FOR PERIODONTAL REGENERATION⁸

Signaling molecules are proteins that may act locally or systemically to influence the growth and function of cells in various manners. These molecules (cytokines) are biological mediators that regulate critical cellular activities including:

1. Mitogenic (Proliferative)
2. Chemotactic (stimulate directed migration of cells); and

3. Angiogenic (stimulate new blood vessel formation)

It includes growth factors, bone morphogenetic proteins, Enamel matrix derivatives etc.

Growth factors

Growth factors are naturally occurring proteins that regulate various aspects of cell growth and development. Various growth factors can be Platelet derived growth factor (PDGF), Fibroblast growth factor (FGF), Insulin like growth factor (IGF), Transforming growth factor (TGF) etc. (Table 3)

Table 3: Effect of various growth factors and BMP in Periodontal Regeneration³

Growth factor	Fibroblast proliferation	Osteoblast proliferation	Mesenchymal cell differentiation	Vascularization	Extracellular matrix synthesis
EGFs	++	-	++	+	-
FGFs	++	++	-	++	-
PDGFs	++	++	-	+ (indirect effect)	-
IGFs	+	++	-	-	++
TGF-β	+ or -	+ or -	-	+ (indirect effect)	++
BMPs	-	±	++	++ (indirect effect)	±

++ greatly increased, + increased, - no or negative effect

Morphogens or differentiation factors (Bone morphogenetic proteins, BMP)

Bone morphogenetic proteins are a group of regulatory glycoproteins that are members of the TGF - β Superfamily. These molecules primarily stimulate differentiation of mesenchymal stem cells into chondroblasts and osteoblasts. In the field of periodontal regeneration, BMP-2 (OP-2 i.e. Osteogenic Protein - 2), BMP -3 (osteogenin) and BMP -7 (OP-1) play very important role.

Platelet Rich Plasma (PRP)

PRP is an autologous concentration of platelets, containing a number of important growth factors such as PGDF, TGF- β , IGF, EGF and VEGF. Additionally PRP also contains proteins (i.e. fibrin, fibronectin, vitronectin) known to act as cell adhesion molecules for osteoconduction and as a matrix for bone, connective tissue and epithelial migration. An average increase of 338% in platelet count is seen during processing which helps in healing process.

Enamel Matrix Derivatives (EMD)

EMD is an acidic extract containing hydrophobic protein assembly of amelogenins which has the capacity to induce regeneration of all periodontal tissues. This is obtained from developing porcine teeth and found to contain TGF- β , & BMP to stimulate bone formation.

Enamel matrix proteins is composed of a number of proteins, such as *amelogenin* (exists in several different sizes), *amelin* (*ameloblastin/ sheathlin*), *enamelin*, *tuft proteins* and *proteases*. Amelogenin being the most abundant component constitutes more than 90% of the matrix.

P-15: (Dentsply, Tulsa Dental Specialties)^{9,10} PepGen P-15 putty is a synthetic P-15 peptide bound to a natural form of hydroxyapatite, in a sodium hyaluronate carrier. It is proven clinically and statistically superior to both DFDBA (Demineralized freeze dried bone allograft) and ABM (Anorganic bone

matrix). ABM/P-15 is a combination has shown the capacity to encourage substantial clinical fill of periodontal infrabony defects.

DELIVERY APPROACHES OF GROWTH FACTORS

It involves direct delivery of growth factors at wound site via carriers or via delivering growth factors gene which results in higher and more constant levels of protein production and help in regeneration of tissue.

It involves –

- 1) Non covalent immobilization
- 2) Covalent immobilization.
- 3) Gene based therapy and cell based approaches.

In first two methods, growth factors are immobilized through non-covalent or covalent binding to a carrier matrix for localized growth factor delivery.¹¹

In gene based therapy, localized application of genes that codes for specific growth factors to produce desired growth factor at a specific tissue site is used. Gene delivery can be performed either by directly introducing the delivery vector into the anatomical site (in vivo) or by harvesting cells from the patient, transferring the gene(s) to the cells in tissue culture and then transferring the genetically modified cells back into the patient (ex vivo).^{12, 13}

STEM CELLS AND THEIR ROLE IN PERIODONTAL REGENERATION (CELL BASED THERAPY)¹⁴

Stem cells are the foundation cells for every organ and tissue in the body. Stem cells are immature progenitor cells capable of self renewal and multi – lineage differentiation through a process of asymmetric mitosis that leads to two daughter cells, one capable of differentiation into more mature cells (progenitor cells).

Seo et al (1993) 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 & 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.

CELL SHEET ENGINEERING¹⁵

This is a recently discovered technology for regeneration of tissues. This technique is superior to the conventional technique as it involves detachment of cultured cells without using enzymatic approach. Cell sheet engineering by use of temperature-responsive dishes provides a novel strategy to produce tissues without a specific scaffold. The resulting cell sheets retain their original extracellular matrix and cell-cell contact. *Okano et al (1995)¹⁹* utilized change in cell culture temperature and a surface-grafted temperature-responsive polymer named poly N-isopropylacrylamide (PIPAAm) to control cell-surface adhesion.

Cell sheet engineering is commercially available under the name of UpCell™ (Cell Seed Inc., Tokyo, Japan)

FUTURE DIRECTIONS

Tissue engineering is emerging as a vibrant industry with a huge potential market. In future, periodontal therapies will involve nano-science and moldless manufacturing technology commonly known as rapid prototyping (RP) or, solid free form fabrication (SFF). These innovations will make it possible to fabricate complex scaffolds that mimic the different structure and physiologic functions of natural fibro-osseous tissues, including those, such as periodontium, which consists of hard and soft tissues. It may also be possible to produce patient specific cell scaffold constructs with

optimal distribution of cells and high vascular permeability.

CHALLENGES WITH TISSUE ENGINEERING:

1. Structural and functional complexity of the Periodontium needs right combination and dosage of growth factors for successful regeneration.
2. Sustained storage and delivery of growth factors with a suitable carrier system is needed for long term and profound effect and promising regeneration of periodontal tissues.

SUMMARY

Tissue engineering constructs and growth factor delivery approaches will inevitably provide a powerful future therapeutic alternative to manage damaged periodontal tissues. Whether this technique progresses to clinical practice or not will depend on concerns over safety, predictability, degree of control, cost etc.

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