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Tissue engineering: A review

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Abstract
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.
The goal of tissue engineering is to promote healing, and ideally, true regeneration of a tissue's structure and function, more predictably, more quickly, less invasively, and more qualitatively than allowed by previous passive techniques.

Keywords: tissue engineering, periodontal regeneration

Introduction
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 [1].
It was proposed by 1993 Langer *et al.* in 1993 as a possible technique for regenerating lost periodontal tissues [2].
The goal of tissue engineering is to promote healing, and ideally, true regeneration of a tissue's structure and function, more predictably, more quickly, less invasively, and more qualitatively than allowed by previous passive techniques [3-4].
The tissue engineering approach to bone and periodontal regeneration combines three key elements to enhance regeneration. (Figure 1)

1. Conductive scaffolds/Extracellular matrix.
2. Stem/Progenitor cells
3. Signalling molecules [5].

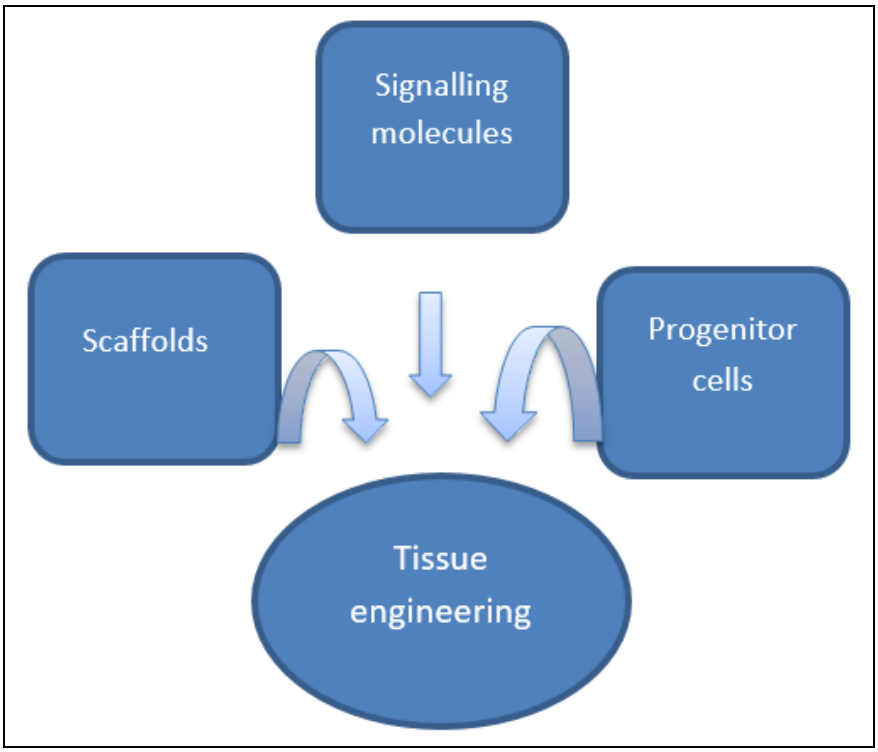

Fig 1: Tissue engineering key elements

Scaffold
The scaffold provides 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 three-dimensional scaffold for the promotion of cellular proliferation and differentiation is critical in periodontal tissue engineering.
The major roles for supporting matrices are listed below

1. It serves as a framework, which maintains the shape of the defect. It provides physical support for the healing area so that there is no collapse of the surrounding tissue into the wound site.
2. It serves as a 3D substratum for cellular adhesion, migration, proliferation and production of extracellular matrix.
3. It serves as a barrier to restrict cellular migration in a selective manner.
4. It potentially serves as a delivery vehicle for growth factors [5].

Biomaterials used as scaffolds

A variety of material has been used in scaffold of tissue engineering. Biomaterials used as scaffolds are described below

Ceramics

Natural and synthetic HA (hydroxyapatite) and beta tricalcium phosphate (TCP) are ceramics used in bone tissue engineering. They are biocompatible, osteoconductive and being protein free, they stimulate no immunological reaction.

Hydroxyapatite was one of the first biomaterial to be used as a scaffold. It may be derived from bovine bone or coralline or made of a pure synthetic material. TCP is a naturally occurring material comprising of calcium and phosphorous and is used as a ceramic bone substitute [6].

Liao *et al.* in a study compared porous beta-tricalcium phosphate/chitosan composite scaffolds with pure chitosan scaffolds. Composite scaffolds showed higher proliferation rate of human periodontal ligament cells (HPLCs) and up-regulated the gene expression of bone sialoprotein and cementum attachment protein. In vivo, HPLCs in the composite scaffold not only proliferated, but also recruited vascular tissue ingrowth; thus, suggesting the benefit of using these composite scaffolds [7].

Polymers

These include synthetic polyesters, such as polyglycolic acid, polylactic acid and polycaprolactone and natural polymers like collagen fibrin, albumin, hyaluronicacid, cellulose, chitosan, polyhydroxyalkanoates, alginate, agarose and polyamino acids.

Synthetic Polyesters

PGA (polyglycolic acid) is a polymer of glycolic acid. It was the first polymeric scaffold used in tissue engineering. It is insoluble in water. It is also used as suture material, and as implants for bone fracture fixation.

PLA (polylactic acid) is the polymer of lactic acid. PLA is more hydrophobic than PGA and more resistant to hydrolysis. Copolymers of PGA have been used for many types of biomaterials, including sutures (vicryl).

PLGA (polylactic-co-glycolic acid) is a copolymer of PGA and PLA. Due to its biocompatibility, controlled structural and mechanical properties, tailored degradation rates, and its potential as growth factor delivery vehicles, it has been considered as the prime candidate for use in regenerative medicine and dentistry.

Natural polymers

Chitosan

It is a biodegradable natural carbohydrate biopolymer that has been shown to improve wound healing and improve bone formation. It is non-toxic and non-immunogenic, and have such structural characteristics that makes it possible to be used as a bone substitute and as a scaffold for cell attachment.

Collagen

Collagen foam

These are fabricated by freeze-drying a solution of collagen and placed in a mold of desired configuration. After physical or chemical cross-linking of sufficient intensity and duration, foam scaffolds become resistant to contraction by tissue cells and exhibit decreased or increased resistance to breakdown by collagenase, depending on the cross-linking regimen [8].

Collagen fiber Fibers with diameters of 300-nm and above have been made on a commercial scale. They can be formed into wools by tangling in a scanning electron micrograph of the wool, into which cells are easily seeded. When cross-linked by methods that do not alter the native 67-nm cross-banding, the fibers are considerably more resistant to collagenase than are foam or gel scaffolds.

Collagen membrane scaffolds Collagen membranes can be prepared by allowing collagen in solution to dry on a surface to which it will not bind, like Teflon or polyethylene. To promote formation of fibrils the solution is neutralized and warmed to 37ºC, allowing the collagen to polymerize and form the fibrils. Before it begins to gel, the solution is spread on a suitable surface and allowed to dry. Membranes may be cross-linked by a variety of methods to improve their wet strength. For example, aldehydic cross-linking will prevent cell attachment, and UV cross-linking will reduce resistance to collagenase [9].

Stem/progenitor cells

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 identical to the stem cell (daughter stem cell) and one capable of differentiation into more mature cells (progenitor cells) [10].

Stem cells may be:

1. Totipotent, i.e. early embryonic cells (one to three days from oocyte fertilization), which can give rise to all the embryonic tissues and placenta.
2. 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).
3. 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) [11].

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 [12-14].

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 [15].

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 and hold great potential for autologous cell-based therapy [14].

Another important characteristic of MSCs for regenerative medicine is their potential allogenic use without immunosuppressive therapy [16]. Within the sphere of periodontal tissue engineering, mesenchymal derived cells have been applied for simultaneous regeneration of the attachment apparatus components.

Signalling molecules

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);
- Angiogenic (stimulate new blood vessel formation) effects [17].

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 is platelet derived growth factor

Platelet derived growth factor

Kohler and Lipton (1974) and Ross *et al.* (1974) discovered that the material released from platelets is the principal source of mitogenic activity present in serum, and is responsible for the growth of many cells in culture that are serum dependent. This activity was later localized to the alpha granules within platelets by Witte *et al.* 1978, Kaplan *et al.* 1979 and called platelet derived growth factor (Ross and Vogel, 1978). PDGF is a dimeric molecule comprising of two peptide chains termed as A and B chains. It is considered to be a potent mediator of periodontal tissue regeneration. It is chemotactic and mitogenic for periodontal ligament cells. It stimulates gingival fibroblast hyaluronate synthesis, a pre requisite for the formation of large aggregate of proteoglycans that provide the lattice for extracellular matrix. Furthermore, alkaline phosphatase activity and osteocalcin are down regulated by PDGF, thereby enhancing bone and cementum formation.

Samuel E. Lynch (1991) [18] demonstrated that short term application of the combination of PDGF-B and IGF-I can significantly enhance the formation of periodontal attachment apparatus during early phases of wound healing following surgery.

Moon-II Cho (1995) [19] conducted an animal study on beagle dogs and introduced the "PDGF modulated guided tissue regeneration therapy". He concluded that PDGF-BB modulated therapy promotes periodontal regeneration more rapidly and effectively as compared to GTR alone. Recombinant human platelet-derived growth factor-BB homodimer is approved for the treatment of periodontal defects and is commercially available as Gem-21 (Osteohealth, Shirley, NY).

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 [20]. Insulin like growth factor-I is found in substantial levels in platelets and is released during clotting along with the other growth factors

Matsuda *et al.* (1992) [21] demonstrated the mitogenic effects of IGF-I on periodontal ligament fibroblastic cells and concluded that a synergistic effect results from using a combination of PDGF-AB and IGF-1.

Transforming growth factor

TGFβ is found in highest concentration in bone and platelets. TGF-β is a strong promoter of extracellular matrix production. It selectively stimulates periodontal ligament fibroblast proliferative activity. It stimulates type I collagen, fibronectin and osteocalcin biosynthesis, as well as bone matrix deposition and chemotaxis of osteoblast. On the other hand, TGF-β decreases synthesis of metalloproteinases and plasminogen activator, and also increases the synthesis of tissue inhibitor of metalloproteinases and plasminogen activator inhibitor, thus resulting in the decrease of connective tissue destruction. It may act as bone coupling factor linking bone resorption to bone formation [22]

Fibroblast growth factor

Fibroblast growth factor is the member of heparin binding growth factor family. There are 7 forms of fibroblast growth factor. Besides its name its activity exists beyond that of fibroblast and includes a wide variety of cell types such as smooth muscles, endothelial cells, chondrocytes and osteoblasts. It has a profound effect on periodontal soft tissue and bone healing as it is mitogenic for fibroblasts, chondrocytes, osteoblasts, smooth muscle. FGF also stimulates, DNA synthesis, angiogenesis, and cell replication [23].

Bone morphogenetic proteins

Bone morphogenetic proteins (BMPs) are the members of transforming growth factor-β superfamily, which play a crucial 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 [24].

They trigger cellular effects by way of heterotetrameric serine/ threonine kinase receptors and intracellular signaling proteins known as small "mothers against" decapentaplegic (Smads) [25].

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.

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 [26].

Clinical applications of tissue engineering for periodontal tissue regeneration

Guided tissue regeneration

Nyman and Karring, in 1982 were the first to have proposed the use of guided tissue regeneration for periodontal regeneration, which marked the evolution of periodontal regeneration technologies using tissue engineering.

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 a favorable niche to guide the right type of cells (PDL cells, cementoblasts, and osteoblasts) to attach at the root surface, and tries to exclude undesirable cells from attaching to the root surface [27].

Protein based approaches [28-29]

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 β;
- Bone morphogenetic proteins;
- Basic fibroblast growth factor;
- Platelet derived growth factor.

Enamel matrix derivative

The rationale for the clinical use of enamel matrix derivative is the observation that enamel matrix proteins are deposited onto the surfaces of developing tooth roots before cementum formation [30].

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 [31].

Use of Enamel matrix derivative (EMD) and a demineralized freeze dried bone allograft (DFDBA) have been demonstrated to be osteopromotive in nature; thus, resulting in an additional increase in bone formation [32].

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 with enhanced 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% [33].

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 [34].

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 molecules in a highly concentrated pure and matrices 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) [35].
- rhPDGF-BB (with β tricalcium phosphate) [36].
- rh BMP-2 (with type I collagen sponge) [37].

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 [38].

Cell based approaches

Cell transplantation using autologous cells is expected to play a central clinical role in the future. Dental cell seeding attempts have attempted to regenerate the periodontal tissues since 1990s. 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 [39].

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.

Challenges with tissue engineering

- Structural and functional complexity of the periodontium needs the right combination and dosage of growth factors for successful regeneration
- 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.

Conclusion

The regeneration of the periodontium is known to be challenging to the clinicians. Thus, the development of new therapies tissue engineered scaffolds opened a new era of the periodontal regeneration. In the near future along with conventional therapy these newer approaches will be useful for regenerating lost tissues and may become key in regenerating oral function disrupted by periodontal disease. The regeneration of the periodontium is known to be challenging to the clinicians. Thus, the development of new therapies based on cells and/or tissue engineered scaffolds opened a new era of the periodontal regeneration.

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