Tissue Engineering and Regeneration Therapy: A Boon for Periodontal Research

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Abstract

Complete regeneration of the periodontium is the aim of any successful periodontal treatment. Recent Technologies have come up with many advances like cell-based therapy, peptides, genetic transfer, scaffolds, bone anabolics, and above all lasers. Four basic elements are required for periodontal repair and regeneration that includes adequate blood supply and wound stability, a source of bone and ligament forming cells, a supporting scaffold, and growth factors to regulate cell migration, synthesis and angiogenesis for revascularization of the site. The newer technologies not only offer unique opportunities but also enhance the predictability of regenerative procedures.

Keywords: *Periodontal Regeneration, Research, Tissue Engineering.*

Introduction

Chronic periodontitis is characterized by irreversible loss in connective tissue attachment and supporting alveolar bone. According to national surveys, more than 30% of adults suffer from moderate type of periodontitis, while severe generalized periodontitis affects almost 15% at some pointin time.[1] Scaling, curettage and open flap debridement are conventional treatment modalities that can control inflammation and lead to the long junctional epithelium formation but there is no regeneration. They have also improved the quality of life of individuals.[2] The main objective of periodontal tissue replacement and reconstruction is to restore mechanical stability and function and also relieve the pain associated with it.[3]

Regenerative Concepts: Melcher 1976, proposed that after flap surgery the curetted surface of roots might be repopulated by different cell types.[4]

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- Cells of Connective tissue in gingiva
- Epithelial cells
- Bone cells
- Cells of Periodontal ligament

The nature of the attachment depends on the cell type that starts populating the root surface after periodontal surgery.

Healing patterns of periodontal tissue damage:

- Down growth of epithelial cells results in a long junctional epithelium.
- Connective tissue might proliferate to cause tissue adhesion and thereby root resorption.
- If the bone cells are predominant,there may be root resorption,ankylosis, or both.
- A completely regenerated periodontium and new cementum can also develop.

How can the lost tissues be ideally replaced?: The concept of tissue engineering is based on the theory that an individual's damaged tissue is better to be replaced by the same natural healthy tissue. This concept has led to the formation of new tissue from preexisting tissues.

Tissue engineering, is a multidisciplinary field that

involves medicine, biology, and engineering. It is likely to revolutionize the quality of life and health of many people worldwide. It can maintain,restore and improve the tissue function respectively. This definition has been broadened recently to include the suitable attempt of tissue regeneration in the body, by the addition of specific biologic mediators,that might be cultured in or outside the body respectively.

Components of tissue engineering:

For the success of tissue engineering three components are needed:

1. To create new tissue: Implanted cultured cell
2. To act as a scaffold to hold cells: a suitable Biomaterial.
3. To form desired tissue type: Biological signaling molecules that can instruct cells.

Cells:

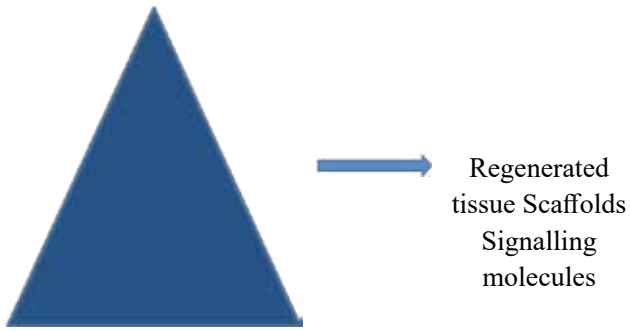

Figure 1. Triad

In vitro: In the laboratory, the construction of a vital tissue can be done and it can be subsequently implanted into the host body. This offers the advantage to examine the tissues as they are being formed and also measure the specific tissues.

In vivo: Properly seeded cell types, implantation of the porous matrix, & signaling molecules can facilitate tissue regeneration. This forms the basis of Tissue engineering.

Strategies to engineer tissues

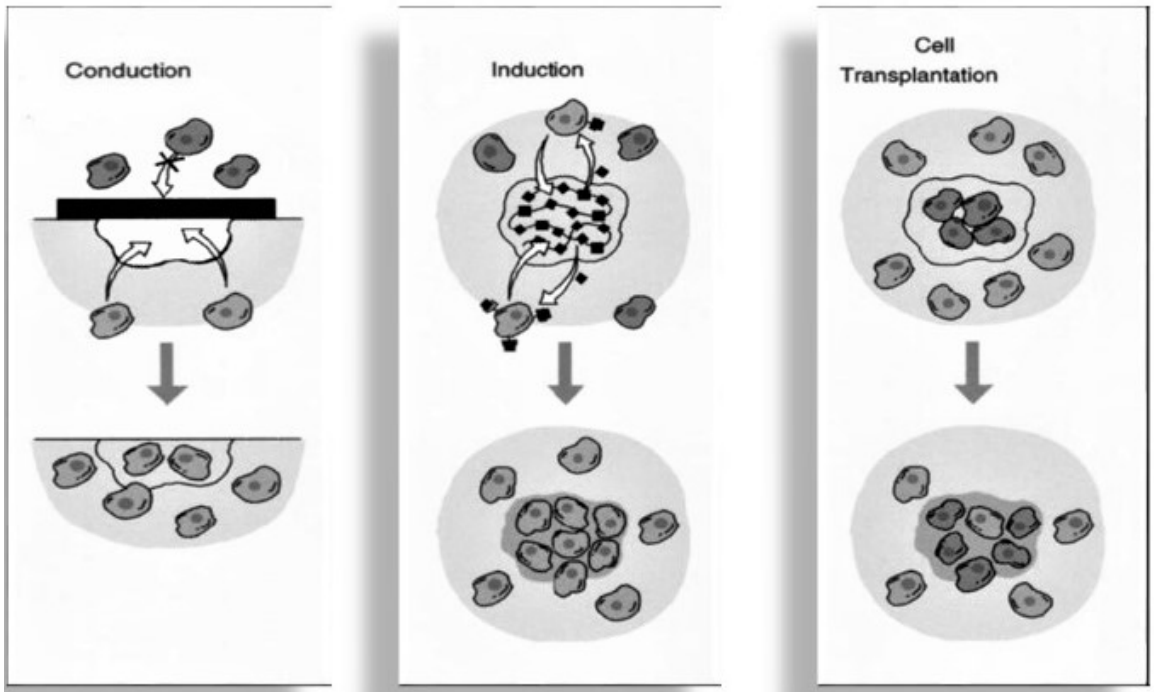

Figure 2. Tissue engineering process

Osteoconduction: "Trellis effect" is a physical effect where the graft matrix forms a scaffold. This scaffold favors cells from outside to enter the graft, thereby forming new bone.

It includes the growth of osteoprogenitor cells, new capillaries, and perivascular tissues from the recipient bone.

Eg: Jaw bone graft, xenograft

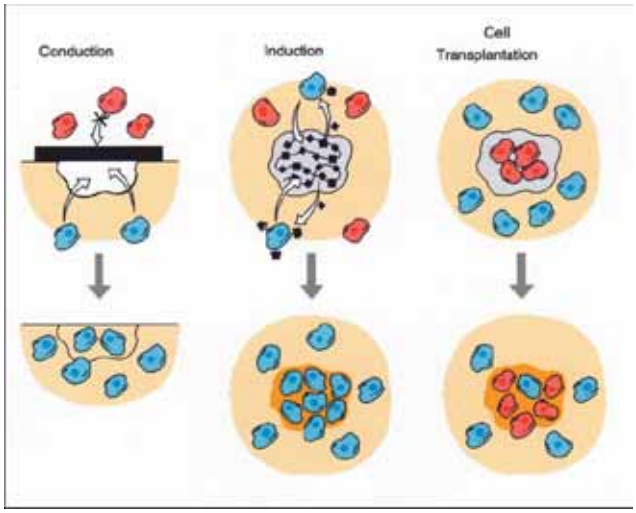

Figure 3. Osteoconduction

Osteoinduction: Biological signals are used to activate the cells close to the defect site.

Bone morphogenetic proteins (BMPs) are the key elements, inducing bone formation.

Limitation:

- It is not possible to know about inductive factors thatare specific for a tissue.
- For the delivery of genes and growth factors to the host site, a biodegradable polymer scaffold can be used as a vehicle.

Advantage:

- The release of growth factors or genes can be controlled based on the polymer breakdown rate.

Cell Transplantation: The cells that are grown in the laboratory are directly transplanted. A vehicle is also used for delivery so that cells and partial tissues can be transplanted to the host site.

Periodontal tissue regeneration:

- Grafts
- GTR
- Growth factors
- Cell sheet engineering
- Enamel matrix protein derivatives
- Stem cell therapy

Advanced regenerative techniques:

- Protein/peptide therapy
- Cell-based therapy
- RNA or gene therapy
- Lasers

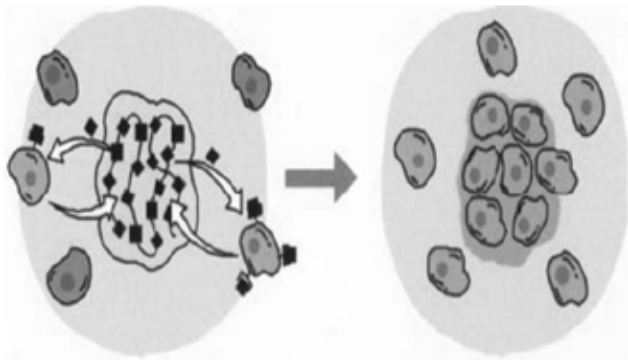

Figure 4. Osteoinduction

Scaffolds: Scaffolds provide and maintain the space that is needed for cell growth and also provide physical support to the process of healing.

Properties of a scaffold:

- To provide a 3D architecture for desired shape volume, and mechanical strength.
- To provide proper characteristics, likeporosity, hydrophilicity and to facilitate tissue infiltration.
- To provide biocompatibility.
- To provide controlled degradation rate coordinated with tissue regrowth.

Used to

- **Guide:** Growth & differentiation of cells
- **Organize:** Physical & chemical signals.

Types of scaffolds:

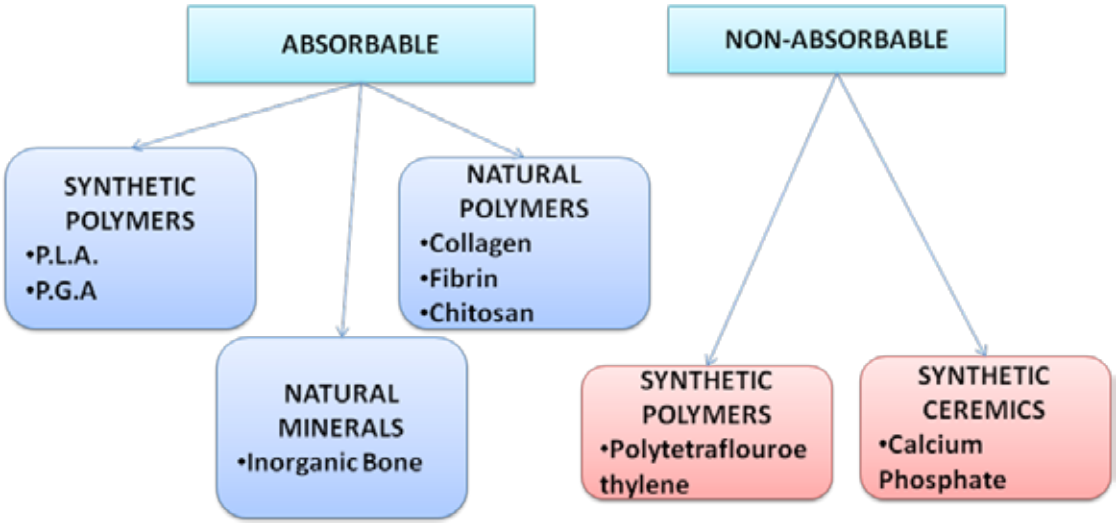

Figure 5. Types of scaffolds

Imaging-based, computer-aided design: According to Park CH 2001 CAD-CAM is one of the most recently developed techniques in scaffold fabrication, that provides a personalized solution for tissue engineering.[5] This technique acquires the 3D anatomic geometry of a defect by high-resolution CT or MRI and that is used as a template for a scaffold. Then 3D bioprinting with desired biomaterials fabricates the scaffold.

Natural minerals or bone grafts:

Bone graft:

Autograft (autologous graft): An organ or tissue transplanted from one site to another in the same person.

Isograft (Isogenic, Syngenic): Graft transplanted from one person to another with identical genetics (eg: identical twins)

Allograft (Homograft): An organ or tissue transplanted from one person to another of the same species but different genus.

Xenograft (Heterograft): A graft transplanted between members of different species (eg: ox bone to humans).

Alloplast: It is synthetic bone graft material or bone graft substitute.

Orthotropic graft: A graft positioned in an appropriate site (eg: bone placed in a bed of bone)

Heterotropicgraft: A graft positioned in an inappropriate site (eg: bone placed in a bed of muscle tissue)

Ideal requisites of bone graft material[6]:

- It should not impede bone growth
- It should enhance revascularization
- It should not elicit immunological responses
- Easy availability.
- Rapid osteoinductive, osteogenetic and. Osteoconductive effect.
- It can be help forming new attachment in periodontal defects.

Limitation of allografts and autografts:

1. There is no excess tissue store in humans for transplantation.
2. While replacing lost bone, donor site morbidity and problems in anatomy, might be there.
3. Immunologic response in allografts due to differences in genes, and can induce transmissible diseases.
4. Synthetic material replacements such as dental implants face a common problem with natural defense mechanisms.

Absorbable synthetic polymers:

They undergo degradation by the process of hydrolysis

Polyglycolic acid - faster degradation

Polylacticacid - this is most stable in-vitro

Polyglactin 910, a copolymer of glycolide and L-lactide – 90/10 molar ratio

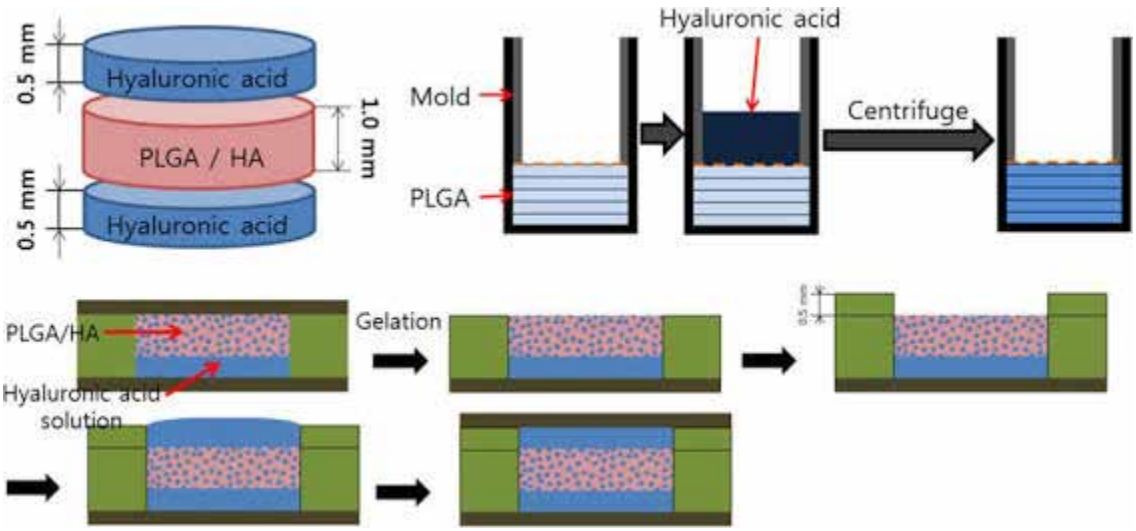

Figure 6: Manufacturing synthetic polymers

Synthetic ceramics(non-absorbable)

These are matrix materials used for facilitating regeneration in-vivo[7]

1. Tricalcium Phosphate:

This is a porous form of calcium phosphate

Ex: ß-TCP

But it undergoes physiochemical dissolution after implantation.

2. Synthetic Hydroxyapatite:

It is a secondary form of bio-ceramic.

Rationale for its use–the naturally occurring mineral in bone is hydroxyapatite

Guided Tissue Regeneration: GTR refers to the placement of a membrane that acts as a barrier between the flap and root surface preventing the gingival epithelium and connective tissues from contacting the root surface during healing.[8]

Design criteria for periodontal GTR devices:

- Easy usage
- Biological activity
- Cell exclusion
- Biocompatible
- Maintenance of space
- Tissue integrity

Barrier membrane for GTR Gottlow's classification:

- First-generation (Non-resorbable membranes)
- Second generation (Resorbable membranes)
- Third generation (Resorbable with growth factors or antibiotics)

Non-resorbable synthetic polymers:

Expanded polytetrafluoroethylene (ePTFE):

- This is the first commercially available barrier for GTR (Gore-Tex)

- Solid nodes and fibrils make itmicrostructure porous.
- The barrier is made of various sizes and shapes to exactly fit periodontal defects in different locations.

Titanium-reinforced PTFE membrane

- Titanium is reinforced between the two e-PTFE layers, thereby a device is formed.
- Facilitates improved space provision and maintenance
- Permits correction of larger defects

Miscellaneous membranes (Millipore membrane, resin-ionomer barrier, rubber dam)

Disadvantages:

- Require a second surgical procedure to remove the membrane and is done usually 3-6 weeks after the intervention when initial healing has occurred
- May interferes with healing by inflicting damage to sensitive regenerated tissue during removal.

Resorbable membranes:

Biomaterials: In cell-based tissue engineering, the expanded cells are seeded onto a scaffold synthesized with the best biomaterial available.

A. Naturally derived materials: Advantage: Along with cellular tissue matrices they offer the advantage of biologic recognition.

B. Synthetic polymers: Advantages: they can be produced in laboratories with the required properties such as increased strength, lowerdegradation rate, and improved microstructure.

Advantages of biomaterials: They mimic the mechanical and biologic functions of the native ECM. The ideal properties of biomaterial arethat it should be biodegradable and bioresorbable. It should also not cause inflammation of the tissue.Incompatible materials can lead to necrosis and foreign body reactions.

Natural biomaterial:

- Collagen (Biomend, Biogide, Biosorb, Ossix plus)
- Glycosaminoglycans (GAGs)
- Chitosan
- Alginates
- Duramater, Cargile membrane

Collagen: It is a major protein of the extracellular matrix, and it has a long triple-stranded helical structure which provides stiffness.

Type 1collagenis found in skin and bone,

Type II foundin cartilage

Type III seen in the blood vessels lumen.

Properties:

- has low immunogenicity
- Is hemostatic, inducing platelet aggregation, which facilitates early clot formation and wound stabilization
- Attracts and activates periodontal ligament and gingival fibroblast cells
- Inhibit epithelial migration

Advantages:

- Lysosomal enzymes sequentially attack and degrade the Collagen implants.
- By controlling the density of the implant and the extent of intermolecular cross-linking resorption rate can be regulated *in vivo.*
- A high rate of implant degradation is due to low density and increased interstitial space for cell infiltration.
- Cell-adhesion domain sequences are seen in Collagen that exhibit very specific cell to cell interactions.

Bio-Mend:

- Is a type I collagen GTR membrane
- Derived from bovine deep flexor tendon
- Is semi-occlusive with effective pore size 0.004 μm
- It is completely absorbed in four to eight weeks.
- Limitation- compromised space maintenance

Artificial biomaterials:

Synthetic polymers:

- Polylactic acid (Atrisorb, Epiguide, Guidor)
- Lactide/glycolide copolymers (Ethisorb, Vicryl mesh)
- Polyurethane
- Polyesters of naturally occurring -hydroxy acids, including PGA, PLA, and PLGA,

Synthetic membranes:

GUIDOR matrix barrier:

- First FDA approved membrane
- It is a double-layered absorbable device made of poly(1actic acid) and a citric acid ester?
- The external layer is designed to allow the integration of the overlying gingival flap.
- Containsrectangular perforations (400-500/cm^2)
- The internal spacers between external and internal layers create a space for the tissue can grow.

ATRISORB barrier:

- It is a poly(DL-lactide) polymer.
- This has been the only approved GTR device to be manufactured chairside.
- This is supplied in a flowable form.
- It can be cut into desired shape and size
- It can be placed into the defect using gentle pressure. Sutures aren't required.
- It is 600-750 μm thick and has properties like adhesion.

VICRYL Periodontal Mesh:

- It is a polyglactin 910 fiber, a copolymer of glycolide and L-lactide (90/10 molar ratio), that is used to prepare a tightly woven meshwork.
- Polyglactin sutures are placed through its coronal margin to anchor it to the tooth.
- Within two weeks of placement, it can lose integrity and resorption is within four or more weeks, depending on the host species.[9]

Polyurethanes membrane:

- These are organic polymers containing the urethane group. According to a study by Warrer et al. 1992 when polyurethane membranes were used for GTR in non-human primates, there was severe inflammation in the margins of the flap,and the recession was also more compared to the poly-(lactic acid) membranes treated tooth.[10]
- Therefore it was not suitable for GTR, as they did not possess all the necessary qualities.

Third generation membrane: They act as a barrier and delivery devices. Growth factors, adhesion factors, antibiotics etc., are released at the wound site.

Subdivided as:

Barrier membranes with Antimicrobial activity: ChengCFet al 2009concluded that the addition of "amoxicillin" or "tetracycline" is addedin GTR membranes,it improves the periodontal ligament cell attachment.[11]

Barrier membranes with Bioactive Calcium Phosphate: This is a three-layered membrane. There are two sides, porous side containing nano-carbonated hydroxyapatite that allow cell in growth and a pure PLGA non-porous side which doesn't allow cells to adhere. These two sides are separated by a transitional layer consisting of nCHAC/PLGA.

Barrier membranes with Growth Factor

Growth factors are proteins. They are naturally occurring and regulate various aspects of cell development.

Rathva VJ 2011 carried a study on bioactive molecules such as different growth factors and enamel matrix derivative (EMD).They concluded that periodontal regeneration is possible.[12]

Functionally Graded Multilayered membranes

Bottino MC 2011 designed FGM. This was fabricated via a multilayering-spinning process consisting of a "core-layer"and two functional surface-layers. [13]

Platelet-Rich Fibrin membrane

First developed in France by **Choukroun** et al.[14]

Characteristics: PRF has a fibrin network and that leads to more effective cell proliferation and migration and thus cicatrization.[15]

Many growth factors, such as platelet-derived growth factor (PDGF) and TGF-β, IGF, VEGF, and thrombospondin,etc., are released from PRF.[16]

Conclusion

Advances have been made in the treatment of complex alveolar bone wounds and periodontal wounds. Be it scaffolds for cell, protein delivery, or gene delivery to specific targeting of signaling molecules, all are in various stages of development. Providing growth factors to the periodontiumis considered as new knowledge revolutionizing the regeneration process.

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