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Periodontal Regenerative Therapy

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ABSTRACT

Periodontal regenerative therapy aims to predictably restore the tooth's supporting periodontal tissues and should result in formation of a new connective tissue attachment (i.e. new cementum with inserting periodontal ligament fibres) and new alveolar bone. Numerous clinical trials have shown positive outcomes for various reconstructive surgical protocols. Reduced probing depths, clinical attachment gain, and radiographic bone fill have been reported extensively for intrabony and furcation defects following scaling and root planing, open flap debridement, autogenous bone grafting, implantation of biomaterials including bone derivatives and bone substitutes, guided-tissue regeneration (GTR) procedures, and implantation of biologic factors, including enamel matrix proteins.

Introduction

Periodontal regeneration is defined as the reproduction or reconstitution of lost or injured part so that the form and function of lost structures is restored. New attachment describes the formation of new cementum with inserting collagen fibres on a root surface deprived of its periodontal ligament tissue(1)Pivotal studies showed only periodontal ligament cells are capable of regeneration(2). A model system developed by Melcher, in 1976, was designed to facilitate selective repopulation of the root surface by cells of the periodontal ligament and alveolar bone.(3) Various bone substitutes have been used in addition to GTR therapy as they facilitate and create the space needed for the regenerative process.(4) Gene based and RNA-based therapeutics, based on the principle of RNA interference are also introduced.(5)

Terminology

Repair(6)

*Repair(*6) Describes healing of a wound by tissue that does not fully restore the architecture or the function of a part.

Periodontal regeneration(6)

Periodontal regeneration(6) defined histologically as regeneration of the tooth's supporting structures, including alveolar bone, periodontal ligament, and cementum over a previously diseased root surface.

New attachment(6)

New attachment(6) is defined as a union of connective tissue or epithelium with a root surface that has been deprived of its original attachment apparatus. This new attachment may be epithelial adhesion and/ or connective tissue adaptation or attachment and may include new cementum.

Re attachment(6)

Re attachment(6) Describes the reunion of epithelial and connective tissue with a root surface.

Biological Foundation of Periodontal Regeneration

In 1976, Melcher (3) suggested that the type of cell which repopulates the root surface after periodontal surgery determines the nature of the attachment that will form. After flap surgery the curetted root surface may be repopulated by four different types of cell, required for periodontal regeneration include:

- epithelial cells to seal the wound area,
- fibroblastic cells for the soft connective tissues of the gingiva and periodontal ligament,
- mineralized tissue-forming cells (alveolar bone cells for bone formation and cementoblasts for cementogenesis), and
- Endothelial cells for forming blood vessels.

Factors Affecting Outcome of Periodontal Regeneration

1. Periodontal Infection: The persistence of poor plaque control, high levels of bleeding on probing in the dentition and persistence of high loads of total bacteria, have all been associated in a dose-dependent manner with poor clinical outcomes(7).
2. Smoking: it significantly impairs regenerative outcomes compared to non smokers. (Tonetti et al 1995)(8).
3. Diabetes Mellitus: poorly controlled diabetes appears to be associated with increased risk of attachment and bone loss (Tervonen et al 1991).(9)
4. Endodontic Status: Jansson et al (1993) (10) has concluded that teeth with coexisting pulpal and periodontal diseases showed deeper probing depths, more advanced radiographic attachment loss

and greater frequency of angular defects, than periodontally involved teeth without pulpal disease.

5. Tooth Mobility: increased mobility influences the severity and rate of progression of periodontal disease.(11,12)
6. Other factors like age, genetics, systemic conditions or stress levels may be associated with sub optimal regenerative outcomes.

Hard Tissue Regeneration

A.Non graft associated techniques

B.Graft associated techniques

Non graft associated new attachment

Removal of junctional and pocket epithelium

The presence of junctional and pocket epithelium

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interferes with the direct apposition of connective tissue and cementum, thus limiting the height to which periodontal fibres can be inserted into the cementum. Several methods recommended for the removal:

***Curettage(*13)**

In this procedure it is necessary that all the epithelium lining the pocket together with the underlying inflammatory tissue as well as the epithelial attachment is removed. The tooth surface is carefully planed and the gingival tissue is carefully readapted to the tooth, a blood clot being allowed to form in the operated area. If the procedure is successful, the blood clot undergoes organization with the production of connective tissue. A layer of cementum forms on the tooth surface while bone deposition takes place on the alveolar side, both surfaces having periodontal fiber insertions. The depth of the crevice will then be reduced at a new location on the tooth, lying occlusally to the point of formation of the new tissue.

***Chemical Agents*(13)**

The most commonly used drugs have been sodium sulfide, phenol, camphor, antiformin, and sodium hypochlorite. However, the effect of these agents is not limited to the epithelium, and their depth of action cannot be controlled.

Ultrasonics and Abrasive stones

They cannot be controlled due to lack of tactile sense.

Surgical Techniques

1. Excisional New Attachment procedure (ENAP)(13): ENAP has been developed and used by the U.S. Naval Dental Corps. It is essentially subgingival curettage performed with a knife. The objectives are to permit thorough soft tissue preparation and to secure better access to the root surface. It is restricted to suprabony pockets whose apical extent lies within the keratinized gingiva. It is not advocated for pockets that extend beyond the mucogingival junction or for treatment of osseous defects.
2. Gingivectomy
3. Modified widman flap

Prevention or Impeding of epithelial migration

It includes Guided Tissue Regeneration therapy. It consists of placing barriers of different types of membranes to cover the bone and periodontal ligament, thus temporarily separating them from the gingival epithelium and connective tissue.

Guided tissue regeneration (GTR)

The concept of GTR is the one that attempts to exclude or prevent this apical migration of epithelium in favor of other cells that will increase the likelihood of regeneration. This method derives from the classic studies of Nyman, Lindhe, Karring, and Gottlow and is based on the assumption that only the periodontal ligament cells have the potential for regeneration of the attachment apparatus of the tooth.(14,15,16)

GTR involves the placement of a barrier covering the periodontal defect in such a way that the gingival tissues (epithelium and connective tissue) are prevented from contacting the root surface during healing. At the same time, a space is formed between the barrier and the root allowing periodontal ligament cells (PDL cells) to produce new connective tissue attachment and bone cells to produce new bone.(14)

Various membranes used in GTR

***Non resorbable membranes* (17)**

Includes

- Polytetrafluoroethylene (PTFE)
- Polyglycolic acid
- Polylactic acid
- Rubber dam
- Resin-ionomer barrier
- Biobrane (knitted nylon fabric mechanically bonded into a semipermeable silicone material)

Polytetrafluoroethylene membrane (PTFE)

The first available material, specially designed for GTR, was made of ePTFE. It is a fluorocarbon polymer with exceptional inertness and biocompatibility. Expanded polytetrafluoroethylne (ePTFE) is PTFE subjected to tensile stress during manufacture, resulting in porous microstructure of solid nodes and fibrils (18). The barrier membrane consists of two contiguous parts. The coronal border allows in growth of connective tissue, hence preventing apical migration of the epithelium, whereas remaining part is occlusive to prevent the gingival tissues outside the barrier from interfering with the healing process at the root surface.

The limitations are that they require a second surgical procedure for removal and there is a susceptibility to risk of latent or post surgical bacterial contamination.

Bioabsorbable membranes

These include Natural membranes : Collagen, cargile, oxidised cellulose, laminar bone and connective tissue graft.

Synthetic membranes: Polylactic acid, polyglycolic acid and polyglycolide-lactide.

Collagen

It has been shown to be chemotactic for fibroblasts, acts as a barrier for migrating gingival epithelial cells, serves as a fibrillar scaffold for early vascular and tissue ingrowth, possess hemostatic properties and is very weakly immunogenic, therefore biocompatible(19).

Synthetic Membranes

The PGA/PLA membrane is composed of a synthetic copolymer of glycolide and lactide. The polymeric omponents of the barrier are broken down by hydrolysis and eliminated from the body through the Kreb's cycle as carbon dioxide and water(20).

There is no additional surgery required, hence reduced patient discomfort and chair side time and related cost. It may elicit inevitable inflammatory tissue reactions that might influence wound healing.

Bone grafts/ bone substitutes (Table 1)

It is based on the assumption that these materials facilitate the regeneration of alveolar bone and root cementum, and create the space needed for regenerative process.(21)

Autografts

Bone obtained from same individual.

Allografts

Bone obtained from different individuals of same species.

Xenografts

Bone from different species.

These graft materials are evaluated based on their osteogenic, osteoinductive and osteoconductive potential.

Osteogenesis

Formation or development of new bone by cells contained in the graft.

Table1. Various graft materials and their effects(4)

Group	Material	Effect	Advantages	Disadvantages
AUTOLOGOUS		Osteogenic Osteoinductive Osteoconductive	Viable cells, growth factors, Intraoralavailability	Rapid resorption potentially inducing root resorptions
ALLOGENIC	DFDBA	Osteoinductive Osteoconductive	Osteogenic potential by release of BMPs	Antigenecity? Infection?
	FDBA	Osteoconductive		-do-
XENOGENIC	Bovine material	Osteoconductive	Similar results as for DFDBA	Poor/ slow resorption
	Coralline	Osteoconductive		Long junctional epithelium, Connective tissue encapsulation.
ALLOPLASTIC	HA	Osteoconductive		No predictable regeneration, long junctional epithelium, connective tissue encapsulation
	b-TCP Bioactive glass Polymers	Osteoconductive		

Osteoinduction

Osteoinduction is a chemical process by which molecules contained in the graft (eg bone morphogenetic proteins) convert the neighbouring cells into osteoblasts, which in turn, form bone.

Osteoconduction

Osteoconduction is a physical effect by which matrix of the graft forms a scaffold that favours outside cells to penetrate the graft and form new bone.

Table 2. Various autogenic bone graft materials

S.no.	Graft material	Sources	Procedure
1.	**OSSEOUS COAGULUM(23)**	Lingual ridge of mandible, exostoses, edentulous ridges, bone distal to a terminal tooth, lingual surface of maxilla or mandible atleast 5mm from the roots.	Bone is removed with a carbide bur #6 and #8 at speeds between 5000 and 30,000 rpm, placed in a sterile dappen dish and used to fill the defect.
2.	**BONE BLEND(24)**		Uses an autoclavable plastic capsule and pestle. Bone is removed from a predetermined site, triturated in a capsule to a workable, plastic like mass and packed into bony defects.
3.	**CANCELLOUS BONE MARROW TRANSPLANTS(24)**	Maxillary tuberosity, edentulous areas and healing sockets.	Edentulous ridges can be approached with a flap, and cancellous bone marrow is removed with curettes, back action chisels or trephine.

Autogenous bone grafts (Table 2)

1. Bone from Intraoral Sites(22): Sources include bone from healing extraction wounds, bone from edentulous ridges, bone trephined from within the jaw without damaging the roots, bone removed from tuberosity and ramus and bone removed during osteoplasty and ostectomy.
2. Bone from Extraoral sites: mainly include iliac cancellous marrow grafts.

Hegedus(1923) pioneered the use of extraoral sites for grafting periodontal osseous defects using tibial bone.

Allografts

Bone allografts are commercially available from tissue banks. They are obtained from cortical bone within 12 hours of the death of the donor, defatted, cut in pieces, washed in absolute alcohol, and deep frozen. The material may then be demineralised, and subsequently ground and sieved to a particle size of 250-750mm and freeze dried. Finally it is vacuum sealed in glass vials.

Types

Freeze dried bone allograft (FDBA)

Demineralised Freeze dried bone allograft (DFDBA) (Table 3)

Xenografts (Table 4)

These are the graft materials obtained from different species. Various materials like anorganic bovine bone, keil bone, ospurum etc. Here the bone is obtained from various animal species and is chemically treated, then used as graft materials.

Table 3. Differences between FDBA and DFDBA (25)

FDBA	DFDBA
Not demineralised	Demineralized
Better space maintenance and slower resorption rate compared with DFDBA	More bone morphogenetic protein expression potential
Osteoconductive	Osteoconductive
More radio-opaque	More radiolucent
Breakdown by way of foreign body reaction	Rapid resorption
Primary indication: bone augmentation associated with implant treatment (eg, guided bone regeneration, sinus grafting, ridge augmentation)	Primary indication: periodontal disease associated with natural tooth.

Table 4. Various xenogenic graft materials

S.no	Graft material	Manufacture	Marketed As
1.	**ANORGANIC BOVINE BONE**(27)	Bone has been chemically treated with ethylenediamine to remove its organic components, leaving a trabecular & porous architecture.	Bio-Oss Bio-Guide
2.	**DESPECIATED BOVINE BONE**(26,21)	The bone is taken from calves, extracted with a detergent and chloroform and methanol, sterilized in propionolactone to remove all antigenic material, sterilized, dried and stored in a container.	Boplant
3.	**OSPURUM**	It is an Ox bone that has been cleansed off all fat, connective tissue and proteins,bone is soaked in potassium hydroxide, acetone and salt solution.	
4.	**KIEL BONE**(24)	Calf bone or ox-bone denatured with 20% hydrogen peroxide, dried with acetone, and sterilized with ethylene oxide.	

Alloplastic materials (28) (Table 5)

An alloplast is a biocompatible, inorganic synthetic bone grafting material.

At present, alloplasts marketed for periodontal regeneration fall into 2 broad classes: ceramics and polymers.

Bioceramics: these includes

- Calcium phosphates (eg, β tricalcium phosphate and hydroxyapatite),
- Calcium sulfate, and
- Bioactive glass

Polymers

They can be natural or synthetic: (Table 6). Natural polymers that have been used in the fabrication of bone grafting materials include

- Polysaccharides (eg, agarose, alginate, hyaluronic acid, chitosan) and
- Polypeptides (eg, collagen, gelatin)

Synthetic polymers

- Polyglycolic acid,
- Poly l-lactic acid,
- Polyorthoester,
- Polyanhydride

Table 5. Various alloplastic graft materials

S.no.	Graft Material	Properties
1.	**HYDROXYAPATITE**	a. Dense HA Osteophilic, osteoconductive, act as biocompatible fillers. Has calcium to phosphate ratio 1.67
		b. Porous HA Obtained by hydrothermal conversion of calcium carbonate exoskeleton of natural coral into HA.
		c. Synthetic (non ceramic) HA Resorbable, low temperature processed, particulate material (OsteoGen).
2.	**b- TRICALCIUM PHOSPHATE**	Porous form of calcium phosphate, has calcium to phosphate ratio 1.5 and exhibit crystal structure of b-whitlockite. Commercially available as Synthograft and Cerasorb.
3.	**BIOACTIVE GLASS(29)**	Composed of silicon dioxide (46 mole%), sodium oxide (24.4 mole %), calcium oxide(26.9 mole%) and phosphorus pentoxide (2.6 mole%). a. PerioGlas (Block Drug Co, Jersey City, NJ) b. Biogran (Orthovita, Malvernm PA)

Biomimetics

PepGen P-15 (Dentsply Friadent, Mannheim, Germany) is bovine-derived hydroxyapatite (anorganic bone) that contains a short polypeptide chain of 15 amino acids, which is a biomimetic cell-binding region of type I collagen. The amino acid peptide, therefore, mimics type I collagen, the major component of bone matrix, promoting cell attachment, which may enhance osteogenesis.

Non bone graft materials (24)

These include sclera, dura, cartilage, cementum, dentine, plaster of paris, plastic materials, ceramics and coral derived materials. None offers a reliable substitute to bone graft materials.

Platelet concentrates (Table 7)

Blood derived products used for prevention and treatment of hemorrhages due to serious thrombopenia of central origin. These can be prepared from donor's plasma and can be used as regenerative materials as they contain growth factors stored in a-granules of platelets (include platelet derived growth factor, endothelial growth factor and transforming growth factor).

Table 6. Polymer materials used as grafts

S.no.	Graft material	Properties
1.	**PROPLAST**	Prepared from two polymer families i.e. polytetrafluoroethylene and pyrolytic graphite. It is of questionable value in correction of osseous defects.
2.	**HARD TISSUE REPLACEMENT POLYMER (HTR POLYMER)**	Non resorbable, microporous biocompatible, osteoconductive material composed of polymethylmethacrylate (PMMA) and polyhydroxyethylmethylacrylate (PHEMA) and calcium hydroxide.

Table 7. Platelet concentrates used as graft materials

S.no.	Platelet concentrate	Method of preparation	Advantages
1.	**PLATELET RICH PLASMA**(30)	Blood is first centrifuged at 5,600 rpm to separate RBCs from platelet poor plasma (PPP) and PRP. It is then reduced to 2,400 rpm to get a final separation of about 30 ml of PRP from the RBCs. PRP is then mixed with bovine thrombin and calcium chloride at the time of application.	Delivers growth factors in high concentration to the site of bone defect or a region requiring augmentation.
2.	**PLATELET RICH FIBRIN**(31)	Blood is centrifuged for 12 min at 2,700 rpm. PRF clot is formed in the middle of the tube. PRF can be obtained in the form of a membrane by squeezing out the fluids in the fibrin clot.	Eliminates the redundant process of adding anticoagulant as well as the need to neutralize it.

Enamel matrix proteins

The enamel matrix proteins, mainly amelogenin, are secreted by Hertwig's epithelial root sheath and induce cellular formation(6). It is approved by FDA and marketed as Emdogain. 90% of the protein in this mixture is amelogenin, with the rest primarily proline-rich non amelogenins, tuftelin, tuft protein, seru proteins, ameloblastin and amelin(32,35).

Recent Approaches to Regeneration

Gene therapy (33)

Several initial attempts to apply gene therapy as a tool for periodontally relevant problems have been explored to date. It is predicted that gene therapy may offer a wider range of treatment options in dentistry than in the past and may become an integral part in dental practice.

Researchers have used several approaches for correcting faulty genes, most common being swapping of abnormal gene through homologous recombination. Other approaches include insertion and repairing of abnormal genes through selective reverse mutation.

Gene transfer techniques (33)

During *in vivo* gene transfer the foreign gene is injected into the patient by viral and nonviral methods.

In contrast an *ex vivo* gene transfer involves a *f*oreign gene transduced into the cells of a tissue biopsy, outside the body, and then resulting genetically modified cells are transplanted back into patient.

Gene therapy is of short lived nature and there is limited quantity of engineered gene that can be delivered.

Tissue engineering and periodontium

It is a contemporary area of applied biomedical research for the fabrication of new tissues to replace damaged tissues and is based on principles of cell biology, development biology, and biomaterials science. Preliminary studies have indicated that periodontal ligament and bone cells can be transplanted into periodontal sites with no adverse immunological or inflammatory consequences.(34)

Conclusion

Over the past 25 years, periodontal regeneration has been the focus of considerable laboratory and clinical research. Indeed, numerous randomized controlled clinical trials have been carried out in order to assess the effectiveness of various surgical techniques aimed at achieving regeneration including guided tissue regeneration, bone grafts etc. A new and promising approach to periodontal tissue engineering has also been reported, that periodontal ligament cells cultured in vitro can cause regeneration after transplantation in animal models.

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