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Bone Regeneration in Periodontal Defects: A Review

Manish Kumar Attavar*, Harkaran Singh Bhullar[1]

1.BDS, Himachal Institute of Dental Sciences, Paonta Sahib, Himachal Pardesh, India.

Corresponding Author: Manish Kumar Attavar BDS, Bapuji Dental College and Hospital, Davangere, Karnataka, India.

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Abstract

The ultimate goal of periodontal therapy is to prevent further attachment loss and predictably restore the periodontal supporting structures that were lost because of disease or trauma in a way that the architecture and function of the lost structures can be reestablished. Conventional nonsurgical therapy and periodontal flap procedures successfully halt the progression of periodontal disease but result in soft tissue recession that leads to poor esthetics in the anterior dentition. Moreover, conventional periodontal therapy often results in residual pockets usually inaccessible to adequate cleaning, which negatively affect the long-term prognosis of the treated tooth. These compromised outcomes can be avoided or minimized by periodontal regenerative procedures that restore the lost periodontal structures. This article presents a brief review of current knowledge of different periodontal regeneration modalities.

Keywords: *Periodontitis, periodontal regeneration; GTR; bone grafts; growth factors; biologics.*

Introduction

Periodontitis is a multifactorial disease characterized by microbially-associated, host-mediated inflammation that results in loss of periodontal attachment, eventually leading to tooth loss **(**1**)**. Periodontitis results in soft and hard tissue destruction around teeth. It has been shown that deep residual probing depths in treated patients represent a risk indicator for the disease progression. In addition, deep residual pockets associated with the presence of intrabony defects or Class-II and Class-III furcation involvements have been strongly associated with increased risk for tooth loss. **(**2**)** The main goal of periodontal therapy is to treat the infection caused by microorganisms in periodontal biofilm and reduce or eliminate further loss of attachment and bone loss, therefore preventing the loss of a tooth. Once the inflammatory aspect of the disease has been controlled, the ultimate goal of periodontal therapy is the regeneration of the destroyed tissues. Periodontal regeneration is a technique that aims to regenerate the damaged tissue around periodontally compromised teeth. Ideally, the treatment protocol for intrabony and furcation defects should result not only in reduction of probing depths, clinical attachment level gain and bone–fill but also the closure of bony defects because of periodontal regeneration **(**2,3,4,5**)**. The regenerative process aims to use scaffolds, cells, and growth factors to enhance biological activity. Current regenerative techniques are aimed at the treatment of intrabony and furcation defects. The focus of this review paper is to present and update the knowledge of periodontal regeneration protocols.

Biologic Foundation

Periodontal regeneration requires new attachment to the root surface, a process that involves the regeneration of periodontal ligament fibres and the insertion of these fibres into newly formed cementum on a root surface that has been exposed previously to periodontal pathogens **(**6,7**)**. It has been shown that cells derived from the gingival connective tissues and the alveolar bone lack the ability to form such an attachment. On the other hand, if the preference is given to repopulation of the root surface by periodontal ligament cells, new connective tissue attachment including new cementum with inserting collagen fibres can be formed. **(**5**)** Hence, the periodontal ligament is of critical importance in the regenerative process.

The concept of “compartmentalization,” in which the connective tissues of the periodontium are divided into four compartments: the lamina propria of the gingiva (gingival corium), the periodontal ligament, the cementum, and the alveolar bone; was developed by Melcher8 in 1976. From this concept of compartmentalization, GTR procedures were developed and barrier membranes were used to accomplish the objectives of epithelial exclusion: cell/tissue repopulation control, space maintenance, and clot stabilization. GTR is based on the exclusion of gingival connective tissue cells and the prevention of epithelial down growth into the wound. By exclusion of these tissues, cells with regenerative potential

(periodontal ligament [PDL], bone cells, and possibly cementoblasts) can enter the wound site first and promote regeneration. Hence, the concept of periodontal regeneration is based on the principle that remaining healthy cells, and or cells attracted to the healing site, have the potential to promote regeneration **(**7,8**)**.

Techniques used for periodontal regeneration

In recent years, various clinical protocols have been shown to enhance periodontal regeneration and improve the clinical outcome in intrabony and furcation defects. This include **(**9,10**)**:

- The use of various surgical techniques in conjunction with the implantation of bone grafts/bone substitutes
- Root-surface conditioning.
- Guided tissue regeneration (GTR)
- Biologics
    1. Enamel matrix derivative (EMD)
    2. Growth and differentiation factors
- Combinations of the above.

Bone grafts

Materials to be grafted can be obtained from the same person (autograft), from a different person of the same species (allografts), or from a different species (xenografts). Bone grafts are generally evaluated based on their osteogenic, osteoinductive or osteoconductive potential **(**9,10**)**.

Objectives of periodontal bone grafting **(**11,12**)**

• Probing depth reduction

• Clinical attachment gain

• Bone fill of the osseous defect

• Regeneration of new bone, cementum, and periodontal ligament

Considerations that govern the selection of graft material **(**11,12**)**

• Biologic acceptability

• Predictability

• Clinical feasibility

• Minimal operative hazards

• Minimal post-operative sequelae

• Patient acceptance

Classification

Bone replacement grafts are classified as follows **(**9,10,11,12**)**:

Human bone

– Autogenous grafts (autografts)

- Extraoral
- Intraoral

– Allogenic grafts (allografts)

- Fresh frozen bone
- Freeze-dried bone allografts
- Demineralized freeze-dried bone allografts

Bone substitutes

• Xenogenic grafts (xenografts)

- – Bovine derived hydroxyapatite
- – Coralline calcium carbonate

• Alloplastic grafts (alloplasts)

- – Polymers
- – Bioceramics

• Tricalcium phosphate

• Hydroxyapatite

– Bioactive glasses

Autografts (12**)** (Autogenous grafts, intraoral sites)

Some authors have reported the presence of a long junctional epithelium between the regenerated alveolar bone and root surface. Thus, the presence of clinical bone fill does not necessarily indicate periodontal regeneration.

Bone obtained from intraoral sites such as:

• Healing extraction wounds

• Bone from edentulous ridges

• Bone trephined from within the jaw without damaging the roots

• Newly formed bone in wounds especially created for the purpose

• Bone removed during osteoplasty or ostectomy.

Autografts (13**)** (Autogenous grafts, extraoral sites)

Autogenous iliac cancellous bone and marrow have been shown to possess a high degree of osteogenic potential. Numerous case reports have demonstrated successful bone fill after application of these materials in furcations and intrabony osseous defects of various morphologies **(**13,14,15**)**.

Bone obtained from sites such as:

- Iliac cancellous grafts

Disadvantages of extraoral autogenous grafts (15**)**

- Postoperative infection
- Exfoliation, sequestration
- Varying rates of healing
- Root resorption

- Rapid recurrence of the defect

Allografts (Allogenic bone grafts)

Several types of bone allografts exist such as iliac cancellous bone and marrow, freeze-dried bone allografts, and decalcified freeze-dried bone allografts. They have the potential to provoke an immune response. Radiation, freezing and chemical treatment has been shown to markedly reduce the antigenicity of allografts. Obtained from cortical bone within 12 hours of the death of the donor, defatted and cut in pieces, washed in absolute alcohol, and deep-frozen. The material may then be demineralized and subsequently ground and sieved to a particle size of 250 to 750 mm and freeze-dried. And finally, vacuum-sealed in glass vials **(**17,18**)**.

Mineralized freeze-dried bone allografts (MFDBA)

Freeze drying may partially distort the three-dimensional presentation of human leukocyte antigens on freeze-dried bone allografts affecting immune recognition. American Academy of periodontology recommends the use of cortical rather than cancellous bone allografts since cancellous bone is more antigenic and there is more bone matrix and more bone inductive components in cortical bone. FDBA is regarded as osteoconductive.

Demineralized freeze-dried bone allografts (DFDBA)

Demineralization in cold, dilute hydrochloric acid exposes the components of bone matrix, closely associated with collagen fibrils, that have been termed bone morphogenetic proteins. FDDBA has superior bone induction properties and clinical studies indicate that sites grafted with this material produce more than 50% bony infill in 78% of sites, compared with only 38% of sites for debridement alone **(**18,19**)**. The material consists of a combination of human freeze-dried powder with human tendon collagen. Following rehydration, it can be layered into a defect and expand to fill it.

Xenografts

Xenogenic materials have also been used for grafting around periodontal defects. These grafting materials are also referred to as anorganic bone, probably because all cells and proteinaceous material are removed during processing. Anorganic bovine bone is the hydroxyapatite skeleton that retains the macroporous and microporous structure of cortical and cancellous bone remaining after chemical or low heat extraction of the organic component **(**20**)**. The advantage of this product is it provides structural

components similar to the human bone with improved osteoconductive capability compared with synthetically derived materials **(**21**)**. Yukna and coworkers have used a natural,anorganic, microporous, bovine-derived hydroxyapatite bone matrix in combination with a cell-binding polypeptide that is a synthetic clone of the 15 amino acid sequence of type I collagen **(**22**)**. The addition of cell-binding polypeptide was shown to enhance the bone regenerative results of the matrix alone in periodontal defects.

Alloplasts

Alloplasts are synthetic, inorganic, biocompatible bone substitutes which promote bone healing. There are presently six types of allo- plasitc materials used in clinical practice which are as follows: nonporous hydroxyapatite (non-resorbable), porous hydroxyapatite or replamineform (non-resorbable), hydroxyapatite cement, beta-tricalcium phosphate (resorbable), HTR (a calcium layered polymer of polymethylmethacrylate and hydroxyethyl- methacrylate, non resorbable) and bioactive glass. The clinical findings appear promising, histologically the grafts tend to be encapsulated by connective tissue with minimal or no bone formation. Microscopic studies have found a limited new bone in proximity to the implanted materials **(**23,24,25**)**.

Technical implications in bone grafting techniques

- Preparation of graft material
- Promotion of a bleeding surface
- Presuturing
- Adequate condensation of graft material
- Fill to a realistic level
- Achievement of tissue coverage
- Placement of periodontal dressing
- Administration of antibiotics

Root Surface Conditioning/ Root Surface Biomodification

The topical application of chemical agents to modify the root surface is among one of the earliest reported clinical approaches to prepare root surfaces for optimal attachment of periodontal tissues and regeneration. Several agents, such as citric acid, tetracycline, and ethylenediamine tetra-acetic acid

(EDTA), have been shown to result in surface biomodification, including detoxification, demineralization, and collagen fiber exposure **(**26,27,28,29**)**. A recent systematic review, however, concluded that chemical root modifiers do not enhance reductions in probing depth or gains in clinical attachment level following periodontal surgery **(**26**)**. Because improvements in clinical measures can occur following periodontal repair via a long junctional epithelium, connective tissue attachment, or both as well as periodontal regeneration, many clinicians continue to routinely apply root modifiers in an effort to promote true regeneration, namely the formation of new bone, cementum, and periodontal ligament. Root surface conditioning with tetracycline or citric acid has been used as a part of regenerative procedures. Root surface conditioning was originally suggested because of the ability of acid to modify the root surface by "detoxifying" it. Root surface conditioning also showed that collagen fibrils were exposed within the cementum or dentin matrix. Recent studies showed that using ETDA, which has a less acidic pH, may also expose collagen fibers and thus promote cell attachment without having a damaging effect on the surrounding tissues. Results from clinical trials using any type of root conditioning agent indicate no additional improvement in clinical conditions **(**30,31,32**)**. Therefore, the use of root surface conditioning as an adjunct to surgical debridement for the purpose of promoting periodontal regeneration is not supported by the literature.

Guided cell repopulation/guided tissue regeneration (GTR)

Guided tissue regeneration describes procedures attempting to regenerate lost periodontal structures through differential tissue responses and typically refers to regeneration of periodontal attachment.

Barrier techniques are used for excluding connective tissue and gingiva from the root in the belief that they interfere with regeneration.

Biologic basis of GTR

GTR has successfully been shown to prevent the migration of epithelial and gingival connective tissue cells into previously diseased root surfaces. The biologic basis of GTR is based on the assumption that the placement of physical barriers prevents apical migration of the epithelium and gingival connective tissue cells of the flap and provides a secluded space for the inward migration of periodontal ligament cells (PDL) and mesenchymal cells on the exposed root surface, which in turn promote periodontal regeneration **(**33**)**. Besides favoring selective repopulation of the wound area, physical barriers are also thought to provide protection of the blood clot during the early phases of healing and to ensure space maintenance for ingrowth of a new periodontal apparatus. GTR membranes, as physical barriers, however, provide no biological effects on differentiation and proliferation of mesenchymal and PDL cells, which is likely to limit their clinical efficacy.

Indications (33**):**

· Narrow two wall / three-wall intrabony defect

· Class II furcation involvement

· Osseous fill around immediate implant placement.

· Repair of osseous defect around failing implants.

· Ridge augmentation.

· Root coverage.

· Repair of apicoectomy defects

Contraindications (33**):**

· Patients with poor oral hygiene.

· Generalized horizontal bone defects

· Class III furcations.

· Multiple adjacent defects.

· In areas of the inadequate zone of attached gingiva

Design criteria for guided tissue regeneration devices (34**)** (Scantlebury)

- Biocompatible
- Cell occlusiveness
- Integration with host tissues
- Clinical manageability
- Ease of use
- For a bioresorbable membrane, tissue reaction resulting from the resorption of the membrane should be minimal.

Properties of an ideal barrier membrane (33,34**)**

1) Should fulfill the occlusive requirements of the GTR principle

2) Should be biocompatible and allow for tissue integrity

3) Should be non-toxic, non-carcinogenic

4) Should be non-antigenic and chemically inert

5) Should be sterile and have the ability to be sterilized easily

6) Should have easy handing characteristics during surgery.

7) Should be sufficiently stiff, so that it does not adhere to the tooth surface

8) Should have designed based on each specific clinical rationale

9) Should have a long shelf life and easily be stored

10) Should preferably be bioresorbable

11) Should

be retrievable in case of complications

12) Should be inexpensive.

Classification of GTR membranes

1) First generation membranes (non-absorbable)

2) Second generation membranes (absorbable)

3) Third generation membrane (1st and 2nd generation membranes with adhesion molecules and growth factors.)

Non resorbable membranes (35**):**

Advantage

- Semi-permeable allowing only the passage of liquids and excluded cell passage.

Disadvantages

- Needs a second surgical procedure to remove it.
- Increased risk of loss of regenerated attachment owing to reentry surgery.
- Increased time, cost and morbidity.

Resorbable membranes:

· Collagen

· Polymers

· Calcium sulfate

· Periosteum, Connective tissue graft, freeze-dried dura mater

· Alloderm

· Lambone

· Gelfoam, Surgicel, Gengiflex

· Cementum impregnated gelatin membrane

· Cargile membranes

· Elastin fibrin matrix

Disadvantages of resorbable membranes (36**)**

· Tissue reaction

· Tendency to collapse toward the root surface

The recommended technique for GTR

The GTR technique for its use is as follows **(**37,38**)**

1. Mucoperiosteal flap raised with vertical incisions, extending a minimum of two teeth anteriorly and one tooth distally to the tooth being treated.
2. Degranulation of the osseous defect.
3. Meticulous scaling and root planning of the root surface
4. Trim the membrane with sharp scissors to the approximate size of the area being treated. The apical border of the material should extend 3 to 4 mm apical to the margin of the defect and laterally 2 to 3 mm beyond the defect; the occlusal border of the membrane should be placed 2 mm apical to the cementoenamel junction.
5. Suture the membrane tightly around the tooth with a sling suture.

6. Suture the flap back in its original position or slightly coronal to it, using independent sutures interdentally and in the vertical incisions. The flap should cover the membrane completely.
7. The use of periodontal dressings is optional, and the patient is placed on antibiotic therapy for 1 week.

Factors Affecting GTR Clinical Outcomes

Regeneration of periodontal defects, although possible, is not always a predictable outcome. Several local and patient-related factors may account for the variability in the clinical responses to GTR. To increase the predictability and clinical success of GTR, factors related to the patient, the defect, and the surgical treatment should be evaluated during treatment planning.

Patient Factors (38)

Patient factors affecting Periodontal Regeneration with GTR Many patient-related factors may adversely affect the healing outcomes after GTR procedures of which smoking, poor plaque control, and residual periodontal disease actively receive special attention, as these can be controlled through behavioral and therapeutic interventions.

1.Smoking: Smoking negatively affects the regenerative outcomes of GTR. Various mechanisms can contribute to the detrimental effects of smoking on healing after GTR, including

- decreased vascular flow,
- altered neutrophil function,
- decreased IgG production and lymphocyte proliferation,
- impaired fibroblast function, and increased prevalence of periodontal pathogens.
- The frequency and duration of smoking inversely correlate to clinical attachment gains after GTR.

2.The level of postoperative plaque control and residual periodontal infection, evaluated by the number of residual periodontal pockets and percentage of sites with bleeding on probing, also affects the clinical responses to GTR

3.Barrier membranes are at a higher risk of becoming contaminated in individuals with high levels of periodontal pathogens and multiple sites with bleeding on probing. Therefore, patients should undergo GTR procedures only after the periodontal infection has been treated.

4.Although there is not enough evidence that diabetes, immunosuppression, and stress impair the efficacy of GTR, it was reported that these patient-related systemic conditions could negatively interfere with the clinical outcomes of GTR.

Local Factors

Case selection is of paramount importance and represents one of the most significant factors in predicting the clinical outcomes of GTR procedures **(**38**)**.

- The presence of cervical enamel projections and enamel pearls interferes with periodontal regeneration and should be removed during regenerative procedures.
- The gingival thickness around the affected area should also be analyzed, as gingival thickness, less than 1 mm is associated with increased prevalence and severity of flap dehiscence over GTR membranes.
- Presurgical tooth mobility has a negative effect on the clinical outcome of GTR and should be controlled through splinting and/or occlusal adjustments.
- Local factors that favor plaque accumulation, such as calculus and overhanging restorations, need to be removed before GTR procedures.
- In addition to these considerations, specific factors related to the regeneration of furcation and intrabony defects should be evaluated.

Complications.

Exposure of GTR membrane

- Most common complication.
- The membrane can get exposed anytime after flap surgery and should not be considered as the failure of the procedure.
- Plaque accumulation, however, becomes a problem. Gentle brushing & chlorhexidine mouthrinses are recommended.

Biologics

According to the Food and

Drug Administration (FDA),

biologics are a wide range of products, proteins, growth factors, or a complex combination of these substances used to treat various diseases or to enhance the regenerative process, as periodontal regeneration, via the activation and stimulation of periodontal cells. Enamel matrix derivative (EMD), recombinant human platelet-derived growth factor-BB (PDGF—BB), and bone morphogenic proteins (BMP) are currently available on the market. **(**39,40,41,42**)**

Enamel matrix derivative (EMD)

EMD (Emdogain-Straumann, Basel, Switzerland) is the most widely studied commercially available bioactive agent used for the purpose of promoting periodontal regeneration. It is derived from the tooth pouches of unerupted porcine teeth and is composed of amelogenins and enzyme components. This material is only sold as Emdogain®. The first study to show the efficacy of this material was conducted by Heiji et al. in 1997, in which EMD was compared to open flap debridement of intraosseous defects **(**43**)**. EMD was associated with significant CAL gains and pocket-depth reduction. Another series of studies compared EMD to GTR with comparable results. The biological rationale for the use of EMD is to recapitulate developmental mechanisms whereby enamel matrix proteins are proposed to play a critical role in stimulating cementogenesis. Based on this rationale, preliminary studies were carried out in animal and human models **(**44,45,46**)**, and histological evidence of regeneration was demonstrated. The defect's anatomy also plays important role in the regenerative potential of this molecule.

Growth factors and differentiation factors

Platelet-derived growth factor (PDGF)

The use of polypeptide growth factors has been proposed based on their ability to promote a variety of cellular functions that are associated with wound healing, including migration, attachment, proliferation and differentiation. The disadvantage is in its handling, which requires the use of scaffold and fillers. PDGF has also been used in association with allogenic bone grafts and shown to induce substantial attachment gain and probing depth reduction in case reports on the treatment of Class II furcation and intrabony defects. Several studies have evaluated the use of this molecule as an adjunct to Beta-tricalcium phosphate (B-TCP), EMD, and bone allograft, with positive results for EMD and allograft. PDGF has been characterized as a "competence factor", which means that it makes a cell competent for cell division; a "progression factor", such as IGF-1 or dexamethasone, is then necessary to induce mitosis, although in some osteoblast and periodontal ligament cell cultures PDGF alone stimulates proliferation. **(**46**)**

Bone Morphogenic Proteins (BMP's)

BMP are proteins found in bone and showed bone regeneration in an animal model. The most studied forms of this type of molecule are BMP-2, BMP-6, and BMP-12. Interesting data came from a study by Wikesjo et al. in a canine model, where the use of BMP-12 showed a regenerated and well-oriented periodontal ligament with newly formed bone and cementum **(**42**)**.

Complications associated with the use of these molecules include:

- Possible ankylosis
- Root resorption

Platelet Rich Plasma (PRP)

PRP is a platelet concentrate that contains a number of different growth factors including PDGF, TGF-β and IGF that have been shown to exert a positive effect on periodontal wound healing. PRP has the advantage of being able to be prepared chairside and safety issues are minimal. There are no randomized controlled clinical trials evaluating the clinical effect of PRP alone in periodontal regeneration. However, the use of PRP combined with several types of grafts for the treatment of intrabony defects resulted in contradictory results ranging from a significant enhancement of clinical attachment gain to no gain.

Future perspectives

Tissue engineering

In light of the clinical unpredictability of currently available surgical techniques to treat all types of periodontal defects, it would appear that these approaches are too simplistic to facilitate the coordinated wound healing events required for the regeneration of a complex organ such as the periodontium. Consequently, a tissue engineering approach has been proposed, whereby periodontal tissues would be constructed in the laboratory under controlled conditions and then surgically implanted into defects. **(**47 In principle, evidence for the viability of this approach has been demonstrated in animal studies showing that autologous cultured periodontal cells can support regeneration. This approach is further supported by evidence that periodontal ligament cells have stem cell properties **(**47,48,49**)**.

A new and promising approach to periodontal tissue engineering involves using periodontal cell sheets prepared *in vitro* and subsequently transplanted into periodontal defects. It has been reported that

periodontal ligament cells cultured using this cell sheet technique can regenerate periodontal ligament tissues after transplantation in animal models **(**50,51**)**.

Gene therapy

One of the major drawbacks related to the use of biologically active agents, such as growth factors, is their short biological half-life which results in their rapid degradation following application. Gene therapy can be used to facilitate extended local delivery of growth factors by transferring the growth factor genes into the local cell population **(**52,53**)**. Gene delivery of PDGF has been accomplished by the successful transfer of the platelet-derived growth factor gene into cementoblast and other periodontal cell types. Animal studies have demonstrated that gene delivery of PDGF stimulated more cementoblast activity and improved regeneration compared with a single application of recombinant platelet-derived growth factor **(**53,54**)**. Although our understanding of gene regulation of PDGF has improved with experimental gene therapy studies, the safety and efficacy of using gene therapy for regeneration have yet to be fully evaluated.

Conclusion

Periodontitis results in destructive changes in the component hard and soft tissues of the periodontium, culminating in the loss of supporting alveolar bone and periodontal attachment. Horizontal patterns of alveolar bone loss are not amenable to periodontal regeneration with current regenerative therapies, including bone grafts. Vertical or angular bony defects, including furcation defects, are often responsive to periodontal regeneration.

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