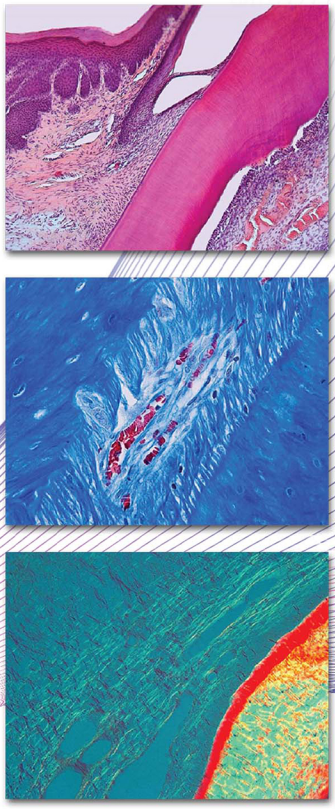
Guided Tissue Regeneration

Procedures, Health Effects and Long-Term Outcomes



Luciano Tavares Angelo Cintra
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GUIDED TISSUE REGENERATION

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LUCIANO TAVARES ANGELO CINTRA
EDITOR

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PREFACE

Regeneration is the reproduction or reconstitution of a lost or damaged tissue through the formation of a new one that can reproduce the form, structure and function of original tissues. Tissue regeneration is a complex process that needs a sequence of molecular events, such as cell adhesion, migration, multiplication and differentiation.

Tissue engineering is an interdisciplinary science that applies the principles of engineering and biological sciences in order to develop biological substitutes for tissues and injured and/or lost organs. In the medical field, these techniques are already used and are widely established. However, they have been used most recently for concepts of tissue engineeringin dentistry. The success of science depends on three basic pillars: cell responsiveness (not necessarily stem cell-based); molecular induction (protein structures that are capable of inducing cellular response) and scaffolds (structures that mimic the extracellular matrix and serve as a support for cell growth).

The Guided Tissue Regeneration (GTR) is a technique used in dentistry that also aims at tissue and bone regeneration, or to repair damaged tissue. It is based on the perception that tissues, for the most part, are capable of self-reconstitution if appropriate conditions are provided. GTR therapy, which was introduced in the 1980s, has been widely used to regenerate lost tissues from periodontal disease, such as the periodontal ligament and alveolar bone. GTR therapy has also been used in the apical

surgeries as a concomitant treatment during the management of endodontic-periodontal lesions. In addition, GTR are used in mandibular defects, implants and intra-bony defects.

The main principle that supports the GTR is cellular selectivity; in other words, a barrier is placed upon a bone defect, which can be associated with bone graft, to hinder the cell penetration of epithelial and connective tissues. This protection ensures the necessary time for the periodontal ligament and alveolar bone cells to differentiate, proliferate and migrate to the inner bone defect, promoting tissue repair.

Recently, the literature has shown many types of techniques that may be employed to promote tissue regeneration. Among the main techniques, the use of bone graft materials associated with GTR and, more recently, the application of polypeptide growth factors (PGFs) are used in favor of tissue regeneration. Previous studies have shown that the association between osseous grafting and GTR promote more favorable results when compared with any other techniques alone. It is believed that while a barrier addresses the dynamics of cell migration, osseous grafts will play an active role in promoting the formation of alveolar bone. The placement of a physical barrier over an osseous defect may prevent the faster proliferating oral epithelium and gingival connective tissues from growing into the bone defect, allowing the cells of the periodontal ligament and endosteum to colonize the blood clot and regenerate the lost tissue.

In summary, the use of the GTR technique for the treatment of periodontal lesions, including furcations of more complex lesions, apical lesions, and large bone loss, is now a reality, as it is evidenced by the regeneration capacity of the periodontal tissues.

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Chapter 1

REGENERATION OF THE PULP-DENTIN COMPLEX

João E. Gomes-Filho*, Paulo C. T. Duarte, Carolina S. Lodi, Gustavo Sivieri-Araújo, Rogério de C. Jacinto, Elói Dezan-Júnior and Luciano T. A. Cintra
Department of Endodontics, São Paulo State University (Unesp), School of Dentistry, Araçatuba, São Paulo, Brazil

ABSTRACT

Complete restoration of a functional pulp-dentin complex cannot be achieved by conventional endodontic treatment. However, with the advent of regenerative medicine, it became possible to regenerate pulp and dentin through alternative endodontic regenerative procedures. These procedures are based on the principles of tissue engineering that aim to develop new tissues to replace lost or malfunctioning organs using a source of stem cells, a three-dimensional scaffold for the growth of these

* Corresponding Author address: João Eduardo Gomes-Filho, Department of Endodontics, São Paulo State University (Unesp), School of Dentistry, Araçatuba, São Paulo, Brazil, R. José Bonifacio, 1193 Araçatuba- São Paulo, Brazil. Tel.: +(0055) 18 36363253, Fax: +(0055) 18 36363253. Email: joão@foa.unesp.br.

cells, and signaling molecules. The most common endodontic regenerative procedure uses blood clots and cell transplantation enriched with or without platelet-rich plasma or stem cell enrichment. Revascularization in humans by using blood clot has been reported in several papers but pulp regeneration has been achieved only in dogs, using stem cell transplantation. The clinician performs blood clot technique relatively easily. However, cell cultivation and expansion have been linked to reduced viability, selection, and undesirable reprogramming and/or cellular dedifferentiation. In addition, it is expensive, time-consuming, and associated with an increased risk of infection. Moreover, as the pulp diminishes with age, alternative sources of stem cells, such as bone marrow, apical papilla, deciduous teeth, periodontal ligament, and adipose tissue need to be evaluated. The aim of this chapter is to highlight the tissue engineering principles relevant to the pulp-dentin complex regeneration using stem cell transplantation and/or blood clot strategies.

Keywords: pulp biology, pulp-dentin complex, regeneration

1. INTRODUCTION

The pulp tissue is a connective tissue of mesenchymal origin, presenting unique characteristics: it is contained inside the inextensible walls represented by dentin and is surrounded by specialized cells named odontoblasts. The intimate relationship between dentin and pulp either by contact or by embryological origin, carries the name pulp-dentin complex (Figure 1).

Complete restoration of functional pulp-dentin complex cannot be achieved by conventional endodontic treatment [1, 2]. However, with the advent of regenerative medicine, it has become possible to regenerate pulp through alternative endodontic regenerative procedures (ERPs) [3]. These procedures are based on the principles of tissue engineering that aim to develop new tissues to replace lost or malfunctioning tissues and organs using a source of stem cells, a three-dimensional scaffold for the ingrowth of these cells, and signaling molecules [1, 3]. The most common ERP uses blood clots and cell transplantation. In a number of studies, the induction of a clot alone did not promote pulp regeneration in immature necrotic dog

teeth [4-6], prompting the investigation of new ERPs that modify the clot or do not use it at all.

Treatment of non-vital immature teeth, especially those with periapical pathology, is challenging. Mechanical preparation of a tooth with a large and divergent apex is extremely difficult because the thin and fragile dentinal walls may be fractured by the instruments used to remove the necrotic contents. Finally, the obturation of a system with wide channels requires the manufacturing of an individual gutta-percha cone with a possibility of root fracture during lateral condensation [7].

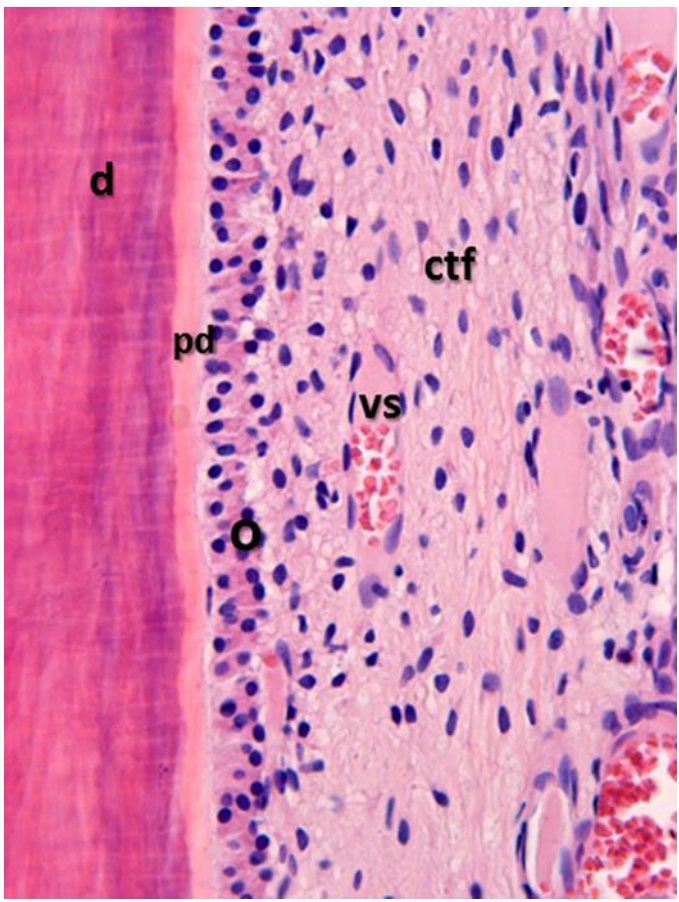

Figure 1. Pulp-Dentin Complex. Note: dentin (d), pre-dentin (pd), odontoblast layer (o), sanguine vessel (vs), and connective tissue fibers (ctf).

The endodontic therapy commonly indicated for those cases, apexification with calcium hydroxide, has several limitations: long period for the formation of mineralized barrier, which is often porous and incomplete [7]; absence of continued root development [8]; and formation of a short thin root susceptible to wall fracture [9, 10]. Another important aspect is the possibility of calcium hydroxide promoting the necrosis of progenitor stem cells in the periapical region essential for regeneration of new tissue pulp or even blocking the migration of these cells into the canal due to the formation of a mineralized tissue barrier [11].

Although many teeth are preserved by conventional endodontic treatment, the ideal treatment would be one in which the inflamed or necrotic pulp tissue is removed and replaced with healthy pulp tissue to revitalize the teeth [12]. An alternative could be to develop and validate a biologically based endodontic procedure that restores functional pulp-dentin complex [7]. The Regenerative Endodontics is the area of dentistry dedicated to creation and implantation of tissues that can replace diseased, missing, or traumatized pulp [12].

Recently, based on new knowledge of stem cell biology and tissue engineering, a method of pulp revascularization has been investigated as a new alternative treatment for immature and necrotic teeth [4, 5, 7, 8 13]. Case reports showed that it is possible to graft immature human teeth and necrotic patients with periapical lesions. In these studies, the teeth were subjected first to disinfection with triantibiotic paste without using root canal instrumentation and subsequently, bleeding into the canals was induced using instrumentation, which resulted in the formation of a clot that was considered a “scaffold” and also a source of growth factors to facilitate tissue repair and regeneration. This treatment method is called pulp revascularization [7, 8, 11, 13, 14, 15]. Our group showed, using a dog as an experimental animal model, that the same results obtained in immature teeth could be achieved in necrotic, mature apex teeth using the method of triantibiotic paste disinfection and clot formation (Figure 2) [16].

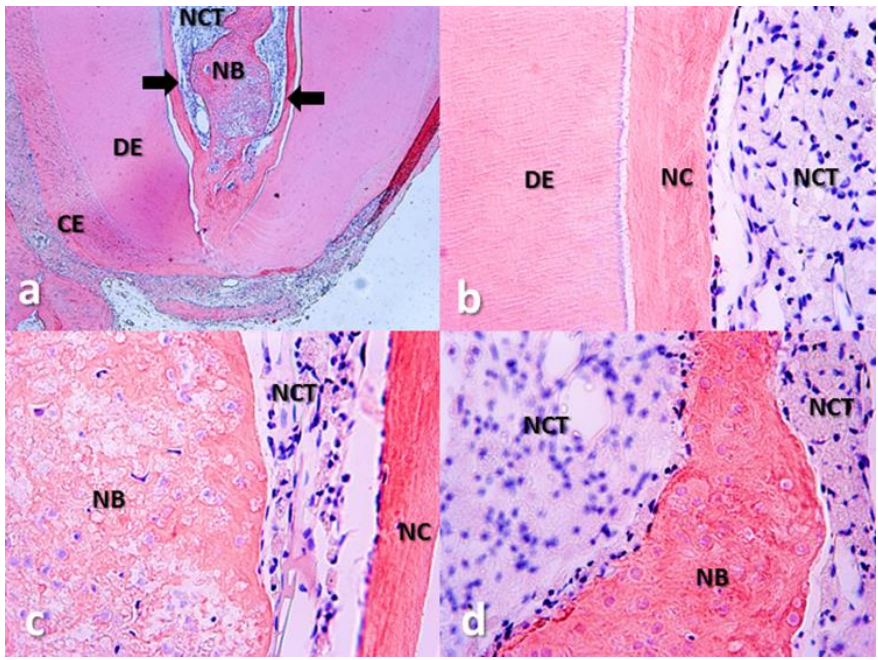

Figure 2. Revascularization after 90 days of inducing bleeding. a. Photomicrograph showing new intracanal tissues at the apical level, highlighting the new connective tissue (NCT) continuous with the new bone-like tissue (NB) and new cement (NC) (black arrowheads) in continuity with the cement (CE) (hematoxylin and eosin stain, 100x) b. Enlarged photomicrograph of new intracanal tissues where the NC is between the NCT and dentin (DE) (hematoxylin and eosin stain, 400x) c. Enlarged shot showing NCT between the NC and NB d. Enlarged shot showing NB in the middle of NCT.

Usage of triantibiotic paste, which has been used in several clinical cases, was proposed by Hoshino et al. in 1996 [17]. It consists of metronidazole and minocycline along with ciprofloxacin as a dressing. The paste is used prior to the induction of bleeding, to create a matrix for the ingrowth of new, vital tissue into the pulp canal space. Our group also investigated the paste and demonstrated its biocompatibility in the subcutaneous tissue of rats, which was found to be similar to that of calcium hydroxide (Figure 3) [18]. However, the paste exhibited dose-dependent cytotoxicty in stem cells from apical papilla [19]. It has been shown to have adequate antimicrobial properties [20].

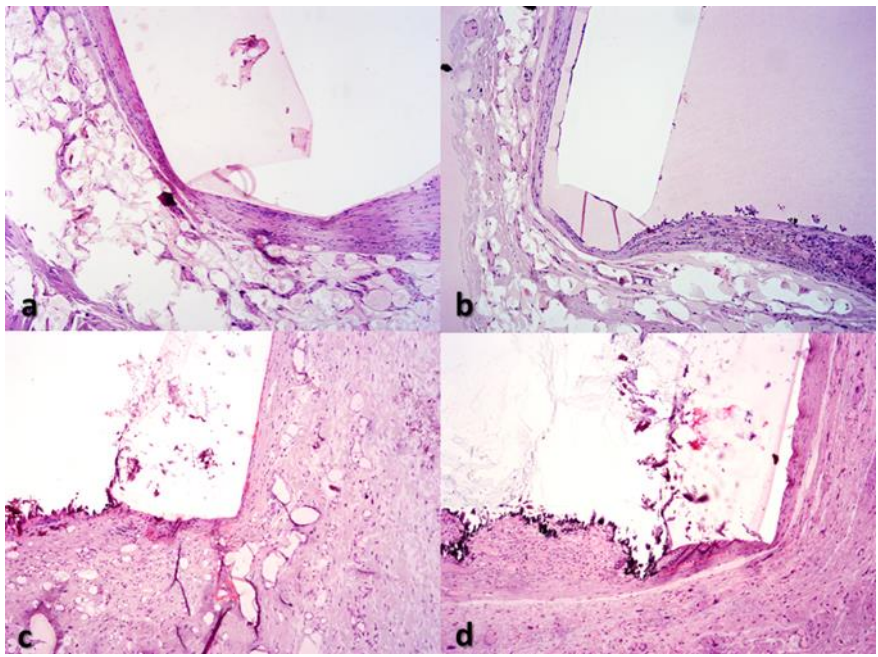

Figure 3. Triantibiotic paste: (a and b) mild inflammatory cell infiltration and reduction in thickness of fibrous capsule (60 and 90 days, respectively; hematoxylin-eosin staining, 100x). Calcium Hydroxide: (c and d) thin fibrous capsule formation, mild inflammatory cell infiltration, and dystrophic calcification in contact with the material (60 and 90 days, respectively; hematoxylin-eosin staining, 100x).

Bacteria-free root canals containing a scaffold for the ingrowth and proliferation of stem cells are crucial for the success of pulp revascularization techniques. The new tissue engineering technologies can decontaminate an infected root canal, implant a scaffold, transplant stem cells, and hermetically seal the coronal access to prevent subsequent infection [21].

Hargreaves et al. 2008 [3], mapped a path for the development of Regenerative Endodontics, highlighting the three components of tissue engineering: cell source, scaffold, and signaling molecules. The source of stem cells for stimulating continued root development can be varied: residual pulp from inside the canal, the apical papilla present in young teeth or bleeding of apical tissues. A suitable scaffold is needed to promote cell growth and differentiation and it should selectively retain the cells,

contain growth factors, and should be biodegradable. Finally, signaling or bioactive molecules such as growth factors are essential to stimulate cell proliferation and differentiation.

1.1. Cellular Source

Most craniofacial structures originate from mesenchymal cells (MCs). During development, the MCs derived from the neural crest migrate, differentiate, and subsequently participate in the morphogenesis of virtually all craniofacial structures, such as cartilage, bone, ligaments, cranial sutures, muscles, teeth and periodontium. They work synergistically with mesodermal cells in the morphogenesis of craniofacial structures. Both mesenchymal and mesodermal cells are derived from embryonic stem cells from the inner cell mass of the blastocyst [22].

Mesenchymal cells undergo asymmetric division. One of the descendants differentiates into the intended cell type and the other replicates as a young mesenchymal cell. These mesenchymal cells, after the completion of morphogenesis, continue to reside in various craniofacial tissues, retaining their stemness. After birth, the MCs are called mesenchymal stem cells (MSCs). In the adult stage, MSCs participate in physiologically necessary tissue renewal. They are recruited to sites of injury or illness, and they differentiate to promote tissue regeneration [22, 23].

Studies suggest that this population of progenitor cells called mesenchymal stem cells (MSCs) present in most mature skeletal and dental mesenchymal tissues, including bone marrow, is able to promote postnatal growth, orchestrating the repair and regeneration. Therefore, all cell therapeutic strategies are based on the assumption that in a specific tissue, in response to molecular signals, a small population of mesenchymal stem cells constitutes self-renewing tissue at the site of transplantation. The conversion and differentiation in this new tissue are dependent on intrinsic biological characteristics of the tissue niche itself. The conversion into a particular lineage is driven by the presence of local morphogenetic factors.

Once the differentiation starts, proliferation is reduced and the biosynthesis of specific tissue proteins begins [24].

Currently, the strategies employed for tissue engineering can be categorized into three classes: conductive, inductive, and cell transplantation. The conductive approach involves the use of biomaterials, which passively facilitate the growth or regeneration of existing tissue. The strategy of inductive tissue engineering involves the activation of tissue near the site of the defect through specific biological signals. Cell transplantation involves using cells grown in the laboratory, depositing them on appropriate scaffolds and subsequently implanting the cell containing scaffold at the defect site [25]. The most commonly used methods in endodontics for pulpal regeneration are through inductive methods and cell transplantation.

1.2. Inductive Method

While considering the inductive approach for the regeneration of pulp tissue inside the root canals, it is important to understand the different sources of stem cells of dental origin, that are available for repair. Young permanent teeth with open apices and necrotic apical periodontitis are not likely to have any vital pulp tissue after infection. However, it was mentioned that in a young tooth, open apex provides good communication between the pulp chamber and the periapical tissues, allowing the occurrence of periapical pathology with a “partially” necrotic and infected pulp [15]. Besides the possibility of survival of stem cells from the pulp tissue, infection of the apical papilla needs to be considered for ensuring pulp regeneration and root maturation. Our group investigated the histopathological condition of the residual tissues after endodontic infection of immature rat teeth and found that vital pulp tissue was observed in the apical third until 60 days and in the vital apical papilla until 90 days of infection (Figure 4) [26]. Such results indicate that the residual pulp from inside the canals may serve as the source of stem cells for stimulating continued root development.

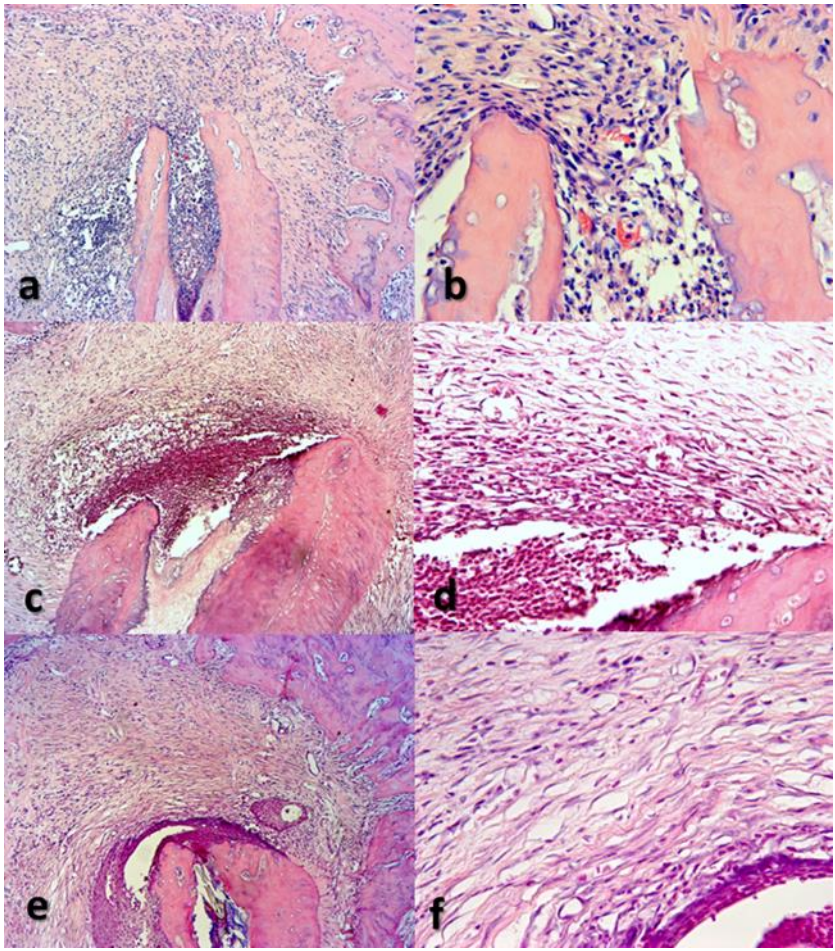

Figure 4. Thirty days of periapical lesion in rats. a. Root showing vital pulp tissue in the apical third of the root (HE, 100x). b. Higher magnification of image a; note apical cell inflammation and apical papilla (AP) (HE, 400x). Sixty days of periapical lesion in rats. a. Root showing remaining tissue in the apical third (HE, 100x). c. Root showing the apical papilla (HE, 100x). d. Higher magnification of image c showing preserved apical papilla with a moderate inflammatory cell infiltration with polymorphonuclear leukocytes (PMNs) (HE, 400x). Ninety days of periapical lesion. e. Periapical region of a root showing the apical papilla (HE, 100x). f. Higher magnification of image e showing mild inflammatory cell infiltration with mononuclear leukocytes MNLs of the apical papilla (HE, 400x).

The recent discovery and characterization of a new population of mesenchymal stem cells residing in the apical papilla (precursor pulp tissue) of immature teeth, called stem cells from apical papilla (SCAP), may help explain why apicogenesis occurs in infected immature permanent teeth [27]. It indicates that these cells might be the primary population of stem cells that give rise to primary odontoblasts responsible for root dentin formation and promote tissue regeneration. The discovery of these cells underscores the important fact that developing tissues may contain stem cells distinct from those in mature tissues [15].

1.3. Blood Clot

In recent case reports describing the revascularization technique in humans, the authors induced apical bleeding through over-instrumentation with endodontic file, forming a clot that was considered a scaffold and source of growth factors to facilitate the regeneration and repair of tissues within the canal [7, 8, 13, 14, 28]. However, there is no histological evidence that a clot is essential for the formation of tissue within the channel. A recent study that compared tissue regeneration in the root canals in a dog model, with and without inducing clot, found no statistically significant difference between the two methods, although the authors suggested the inclusion of the same to maximize the chances of repair [4].

1.4. Platelet-Rich Plasma (PRP)

Although the usage of PRP as a scaffold in regenerative approaches has been reported in the literature [3], it is known that both PRP and platelet poor plasma (PPP) are not scaffolds. Instead, they are components of the scaffold, which should have a three-dimensional structure.

Hargreaves et al. 2008 [3] highlighted the benefits of PRP, which possesses several characteristics of an ideal scaffold; it is rich in growth factors, easy to prepare in the office, can be obtained from the patient's own blood (autologous), breaks down over time, and forms a three-dimensional fibrin matrix. The advantages of its use as an alternative to the creation of a blood clot, are the higher concentration of growth factors and absence of red blood cells, which undergo necrosis after clot formation (red blood cells release hemoglobin, which is toxic). PRP can constitute a three-dimensional structure that can serve as a scaffold, only when activated.

Platelet-rich plasma PRP has been investigated in the context of human ERPs [3]. A patient treated with PRP showed the presence of pulp tissue, but lacked bone tissue [28]. However, no pulp regeneration was achieved by injecting PRP, dental pulp stem cells (DPSCs), or a mixture of both into the roots of mature dog teeth, without inducing a clot [29]. In a human subject, injection of PRP and induction of a blood clot increased the root length and thickness compared to that observed with the induction of a blood clot alone [30].

1.5. Activated PRP (Platelet Gel)

Platelets in the resting state are non-thrombogenic and require a trigger before acting as an active and powerful component in the processes of hemostasis and wound healing. When they are activated by thrombin, they can change shape, developing pseudopodia, which promotes platelet aggregation. Subsequently, they release their granular content, the growth factors platelet (FCPs), which will function during various stages of the tissue healing process. Based on the actions of several FCPs during different stages of the cascade of the repair process, the use of platelet gel (GP) seems to be an interesting proposition, since it has the supreme advantage of having multiple growth factors, which act synergistically to promote mitogenesis of mesenchymal stem cells at the wound site [31].

The alpha granules in the platelets in PRP contain growth factors and their release requires activation of the platelets. Thrombin, the most potent activator of platelets, induces an immediate and dose-dependent release of these platelet growth factors [32]. Bovine thrombin has been used for activating the platelets in PRP, but it has been associated with the development of antibodies (immunogenicity). Alternatively, PRP can be activated with autologous thrombin produced from PPP or PRP prepared from total autologous blood, using commercially available kits [31].

The use of a synthetic peptide with the sequence SFLLRN that mimics thrombin, known as thrombin receptor activator peptide 6 (TRAP), facilitates the sustained release of higher concentrations of platelet growth factors including higher concentrations of platelet derived growth factor-AB (PDGF-AB) and transforming growth factor-β (TGF-β) and reduces the retraction of platelet gel [33].

Mixing PRP and thrombin in calcium chloride, which neutralizes the effect of the citrate anticoagulant present in the donated blood, results in the activation of the platelet concentrate, leading to the formation of a viscous platelet gel (GP) that can be applied with a syringe as a solid mass over the tissues [31].

2. PREPARATION OF PERIPHERAL BLOOD [16]

2.1. Platelet-Rich Plasma Gel (PRP Gel)

Using a disposable 10 mL syringe containing 2 mL of citrate-phosphate-dextrose-adenine-1 (CPDA-1), 8 mL of peripheral blood is collected from the jugular vein (5 mL for preparation of PRP and 5 mL for preparation of autologous thrombin). The tubes are centrifuged initially at 300 *g* for 10 minutes at 22°C. The supernatant is subjected to a second centrifugation at 640 *g* for 10 minutes to obtain PRP. PRP gel can be prepared by activating the PRP with autologous thrombin at a ratio of 2:1.

2.2. Autologous Thrombin

Plasma samples (250 μL) mixed with 10% calcium gluconate (75 μL) are incubated at 37°C for 15 min and then centrifuged at 640 *g* for 10 min, after which the supernatant containing autologous thrombin is ready for use.

2.3. Signaling Molecules

FCPs are peptide growth factors in platelets that promote proliferation, differentiation, chemotaxis and migration of various cells, thus playing an important role in healing processes. These factors include: (i) PDGF-AB, which promotes chemotaxis (attraction of cells to the wound site), mitogenesis, angiogenesis (endothelial mitosis in functional capillaries), and activation of macrophages (tissue debridement and secondary source of growth factors); (ii) TGF-β, which regulates endothelial mitogenesis by stimulating the proliferation of undifferentiated mesenchymal cells, induces their differentiation into fibroblasts and osteoblasts, regulates the synthesis and secretion of collagen, regulates the mitogenic effects of other growth factors, stimulates endothelial chemotaxis and angiogenesis, and inhibits the proliferation of lymphocytes and macrophages; (iii) Fibroblast Basic Factor b (FBFb); (iv) Epidermal Growth Factor (EGF); (v) Vascular Endothelial Growth Factor (VEGF) and (vi) Connective Tissue Growth Factor (CTGF) [31].

Growth factors act in three different ways: paracrine action, in which growth factors secreted by a given cell stimulate the cells in its immediate vicinity; autocrine action, in which the cell releases growth factors that stimulate and increase the activity of the secreting cell itself; endocrine action, in which cells secrete factors that influence a cell with a phenotype different from that of the secreting cell, and which is located at a distant site. Because of these different mechanisms of action, growth factors are capable of affecting different cell types and promoting a number of cellular functions in different tissues [31].

2.4. Cell Transplantation

The simplest method of cell transplantation with appropriate regenerative potential is through injection of postnatal stem cells into the disinfected root canal systems. There are many advantages of the approach using autogenous stem cells. First, autogenous stem cells are relatively easy to collect, can be applied with a syringe, and have the potential to induce the regeneration of new pulp. Second, it is a proven procedure, which is already in use in regenerative medical applications such as bone marrow transplantation. The disadvantages are that the cells may have low survival rate and could migrate to different locations in the body, resulting in aberrant patterns of mineralization. One method of mitigating this would be to apply them along with a fibrin clot or other scaffold that would help maintain the cellular localization [12].

Dog pulp regeneration has been achieved by transplanting stem cells from pulp [12, 34, 35]. However, cultivation and expansion of the cells have been linked to reduced viability, selection, and unwanted reprogramming and/or cellular dedifferentiation [36]. It is expensive, time-consuming, and associated with an increased risk of infection [37]. Moreover, since pulp diminishes with age, alternative sources need to be evaluated [35]. Bone marrow has been used as an alternative source of MSCs for dog pulp regeneration [35]. The use of bone marrow aspirate (BMA) can be a straightforward, low-cost, and fast method with low contamination risk [38, 39].

An endodontic regenerative procedure for mature necrotic permanent teeth has been clinically investigated and the resolution of apical radiolucency and regression of clinical signs and symptoms was observed at recall appointments [40]. Histologic evaluation of transplantation of DPSCs and/or PRP into root canals showed no evidence of pulp tissue or even improved tissue ingrowth in mature vital dog teeth [41].

Pulp regeneration in mature teeth is challenging compared to that in immature teeth, due to the lower amount of stem/progenitor cells present, narrower apical pathways for cell migration, and the difficulty of disinfection. Clot formation creates a three-dimensional fibrin scaffold that

may contain MSCs from periapical tissues [42] and growth factors secreted from platelets, to form the tissue engineering triad [31]. Molecular analysis of the blood from the canal demonstrated a significant presence of stem cell markers CD73 and CD105 compared to that in circulating blood [42]. The stem cells may be derived from peripheral blood, periodontal ligament, bone marrow, granulation tissue, or periapical lesions [43-47]. However, such treatments produced mainly connective, cementum-like, and bone-like tissues, but not pulp tissue, which is in accordance with previous results [4, 5, 6], and can be associated with the ingrowth of periodontal ligament stem cells after mechanical irritation [44]. Moreover, the lack of stem cells derived from dental pulp, particularly the fractionated cells, could have contributed to the absence of pulp tissue. In dogs, when CD105+ stem cells of dental pulp along with stromal cell derived factor 1 (SDF-1) in a collagen scaffold were implanted, complete pulp regeneration was achieved [34]. Similar pulp regeneration was observed in dogs when dental pulp, bone marrow, or adipose tissue-derived CD31- cells were delivered in a collagen scaffold along with SDF-1 [35].

It appears that the inductive method is not able to achieve pulp regeneration, but only pulp revascularization and promoting the formation of connective, cementum-like, and bone-like tissues, not pulp tissue. However, stem cell transplantation has been shown to achieve the pulp regeneration as reported earlier [34, 35]. Pulp regeneration requires the triad of cells, scaffold, and growth factors.

Stem cells are unspecialized cells that continually divide and are characterized by their ability to self-renew and to generate complex tissues and organs. These characteristics distinguish stem cells from progenitor cells or differentiated restricted cells, which have a restricted developmental potential and reduced capacity for proliferation [48].

Many adult tissues such as bone marrow, brain, skin, muscle, and adipose tissue have a subpopulation of stem cells [49, 50]. Stem cells are also found in dental tissues. One of the first types of stem cells related to the tooth was found in the pulp of permanent teeth; these were called stem cells from dental pulp (DPSCs) [51]. Subsequently, stem cells of human exfoliated deciduous teeth (SHED), stem cells from apical papilla (SCAP),

dental follicle progenitor cells (DFPCs), and periodontal ligament stem cells (PDLSCs) have been characterized [27, 44, 52, 53, 54].

Human dental pulp has a subpopulation of cells with phenotypic and genotypic characteristics of stem cells, as demonstrated by their ability to differentiate into a variety of cell types, including neural cells, adipocytes, and odontoblasts [51, 52]. Recent evidence demonstrates the potential to use stem cells from dental pulp for the regeneration of bone, cartilage and dental pulp tissue [54].

Stem cells from human exfoliated deciduous teeth (SHED) are the population of stem cells found in dental pulp of deciduous teeth [52]. It has been shown recently that these cells have the potential to regenerate dental pulp *in vivo* [55]. Stem cells from deciduous teeth are an attractive source of autologous cells for regenerative endodontics, since the trauma leading to necrosis of immature permanent teeth is normally observed during the mixed dentition stage [9].

The use of stem cells for clinical applications will depend on their rate of proliferation, differentiation potential, and accessibility. When DPSCs were compared with bone marrow stem cells (BMSCs), it was observed that the DPSCs showed better odontogenic capacity compared to BMSCs [56]. Both SHED and DPSCs are able to produce a tissue with morphological and functional characteristics very close to those of human dental pulp [57, 58, 59]. However, SHED have the advantage of being extracted from naturally exfoliated teeth, thus making them an attractive option for tissue engineering.

The regeneration of pulp tissue is dependent on the differentiation of stem cells into odontoblasts, which are associated with dentin formation as well as repair of the pulp-dentin complex. Several proteins have been used to identify the processes involved in the differentiation of odontoblasts. Dentin Sialophosphoprotein (DSPP) is a highly phosphorylated non-collagen protein, which is cleaved, immediately after secretion, into two daughter proteins, dentin sialoprotein (DSP) and dentin phosphoprotein (DPP) [60, 61]. DSPP is highly expressed in odontoblasts. It can also be found in osteoblasts at lower levels [60, 61, 62, 63]. Dentin matrix protein 1 (DMP-1) is expressed by odontoblasts during differentiation [64, 65].

The expression of DSPP and DMP-1 by functional odontoblasts in the early stages of odontogenesis is consistent with the hypothesis that both DMP-1 and DSPP play a major role in the mineralization of dentin [64, 66]. Matrix extracellular phosphoglycoprotein (MEPE) is a member of the bone matrix protein family and is involved in the regulation of cellular metabolism during the mineralization processes. Analysis of the expression and function of MEPE in the differentiation of stem cells from dental pulp showed contradictory results [67, 68]. Importantly, none of these markers (individually) can unequivocally demonstrate odontoblast differentiation.

Stem cells require stimulation for their differentiation. Growth factors and morphogenetic factors are proteins that bind to specific cell membrane receptors and initiate a series of signaling pathways that coordinate cell function. These molecules play a critical role during the differentiation of stem cells, resulting in the formation of sense organs and tissues during embryogenesis. The same growth factors may be used therapeutically to guide the cellular processes for the regeneration of tissues and organs. Thus, there is a similarity between the factors that regulate dentinogenesis and the factors that regulate the repair [69].

Growth factors play an important role in the repair process of dentin and pulp [69, 70]. Some of them, such as TGF-β, bone morphogenetic proteins (BMPs), PDGF, fibroblast growth factor (FGF), and VEGF incorporated into the dentin during dentinogenesis, are retained as “fossil” molecules [71, 72, 73]. When released from the dentin, these bioactive proteins are able to induce the cellular response that leads to the formation of tertiary dentin and pulp [70, 74]. The arrangement of tubular dentin facilitates the release of growth factors when dentin matrix is demineralized by exposure to acidic agents of decay or pulp dressing material.

Calcium hydroxide ($CaOH_2$) was able to solubilize dentin and release molecules that regenerate dentin [75]. Calcium hydroxide is one of the most successful pulp dressings capable of inducing the repair of pulp-dentin complex (formation of dentin bridge) [76], and it remains the gold standard with which new test materials are compared [77]. Despite its widespread use in clinical practice for over 60 years [78], the mechanism

of action by which $CaOH_2$ promotes reparative dentinogenesis and dentin bridge formation is still unclear. The effectiveness of $CaOH_2$ has been attributed to the release of hydroxyl ions [76], raising the pH of the exposed pulp tissue. This increase in pH causes local necrosis of the exposed pulp tissue [79]. Thus, the effect of $CaOH_2$ has been considered to be mediated via chemical insult, which causes irritation of vital pulp tissue below the layer of necrosis and stimulates the pulp regeneration processes in some unknown manner. It has been postulated that the alkaline pH maintained in the treated region creates favorable conditions for the formation of dentin. The elevated presence of calcium ions increases the expression of genes promoting mineralization (osteopontin and BMP-2) in pulp cells [80]. The results from the studies of Davidson & Guo, 2000 [81] are also consistent with the role of calcium ions in the formation of reparative dentin, suggesting that they may act alone or in synergy with high pH to form a dentin bridge.

Previous studies have also demonstrated that dentin matrix components solubilized by EDTA exhibited morphogenetic activity and induced reparative dentinogenesis *in vivo* [74, 82]. A variety of chemical agents are capable of solubilizing dentin matrix and these agents include chelators, such as EDTA. Acids are widely recognized to demineralize dentin, as occurs during tooth decay. They were used to solubilize the organic matrix components of dentin during the first studies on the dentin composition [83, 84]. Subsequently, EDTA and other chelating agents have largely replaced acids for the isolation of non-collagen components of dentin matrix by calcium chelation.

Approximately ninety BMPs have been identified so far, some of which (BMP-2, BMP-4, BMP-7, for example) initiated the events that induced dentinogenesis in animal models [85, 86]. These growth factors can be found in dentin matrix and presumably induce differentiation of the stem cells in pulp into odontoblasts.

Recent studies have shown that SHEDS seeded onto dental slice (tooth slice) scaffolds differentiated into odontoblasts, as demonstrated by the decreased rate of proliferation and acquisition of markers of odontoblastic differentiation such as DMP-1, DSPP, and MEPE. On the other hand,

when cultured on scaffolds without cells or dentin or when the slice was deproteinized by prolonged exposure to sodium hypochlorite (NaOCl) [87], stem cells did not differentiate into odontoblasts, as evidenced by the absence of their markers. Treatment of the dental slices with EDTA potentiated the marker expression, thus proving beneficial for regenerative endodontic procedures [59].

FINAL CONSIDERATIONS

Based on current literature, root canal treatment using only disinfection and bleeding to form a clot as scaffold (inductive method) is not enough to regenerate the dental pulp complex. Adding stem cells yielded better results in animal models. However, this method has not been used in clinical practice until date.

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Chapter 2

GROWTH FACTORS IN GUIDED TISSUE REGENERATION

Renata O. Samuel*[*], João E. Gomes-Filho, Gustavo Sivieri-Araújo, Rogério de C. Jacinto, Elói Dezan-Júnior and Luciano T. A. Cintra
Department of Endodontics, São Paulo State University (Unesp), School of Dentistry, Araçatuba, São Paulo, Brazil

ABSTRACT

Advances in tissue engineering have provided excellent results in regeneration. These concepts have been increasingly used successfully in Odontology. Guided tissue regeneration (GTR) involves the use of a membrane to contain the clot at the treatment site and promote regeneration. The objective of current research is to improve the technique by combining biomaterials and bioactive molecules, as well as improving the tissue engineering techniques. Bioactive molecules such as growth factors are capable of inducing morphogenic signals, promoting

[*] Corresponding Author address: Renata Oliveira Samuel, Department of Endodontics, São Paulo State University (Unesp), School of Dentistry, Araçatuba, São Paulo, Brazil, R. José Bonifacio, 1193 Araçatuba- São Paulo, Brazil. Tel.: +(0055) 18 36363253, Fax: +(0055) 18 36363253. Email: re_samuel@hotmail.com.

angiogenesis, differentiation, and cell proliferation. Growth factors are at the center of many studies, but there is no consensus about the advantage of using bioactive molecules or the growth factors to be used in specific clinical scenarios. Bone morphogenic proteins (BMPs), platelet-derived growth factor (PDGF), and peptides of the parathyroid hormone (PTH) have been studied. BMPs have been associated with bone regeneration. However, this characteristic is more evident when BMPs are mixed with a biomaterial that supports the clot. PDGF has been shown to be mitogenic and chemotactic for periodontal ligament cells, with the additional effect of promoting regeneration of bone, ligament, and cement. PTH has been shown to exert multiple anabolic effects on both cancellous and cortical bone. This review makes a comparative evaluation of results from clinical and histological studies using growth factors in animal models and humans.

Keywords: guided tissue regeneration, growth factors

1. INTRODUCTION

1.1. Tissue Engineering and GTR

Tissue engineering is an interdisciplinary science that applies the principles of engineering and biological sciences to develop biological substitutes for tissues and injured and/or lost organs [1]. These techniques are already well established and in use in the medical field [2]. However, the technological concepts of tissue engineering have been making inroads into dentistry more recently. The success of this science is contingent on three basic factors: responsive cells (not necessarily stem cell-based) [3]; inducing molecules (proteins that are capable of inducing cellular response), and scaffolds (structures that mimic the extracellular matrix and serve as a support for cell growth) [1].

GTR is a technique used in dentistry for tissue and bone regeneration or healing damaged tissue [4]. It is based on the perception that tissues, for the most part, are capable of self-healing, if appropriate conditions are provided [5]. GTR, in the initial stages, involved using a membrane to contain the clot at the site of injury to facilitate tissue regeneration [4].

More recently, the trend is to make use of several concepts of tissue engineering to ensure success. In addition to the membrane, in most cases, the use of growth factors has been recommended to stimulate host cell chemotaxis, proliferation, differentiation, and new tissue formation at the site of bone deficiencies [6]. Also recommended is the use of a carrier or scaffold that delivers the growth factors, functions as a cell proliferation guide, and acts as an extracellular matrix [7].

Owing to the rapid advances in research and the increased diversity of biomaterials, there are no well-established protocols for each technique, especially for those that include growth factors. BMPs, PDGF, and PTH are some of the growth factors discussed for use in GTR. This review aims to summarize the function of these growth factors with respect to the clinical status and prognosis.

2. BMPs

BMPs are multifunctional cytokines and considered members of the transforming growth factor-β superfamily on the basis of their amino acid sequence homology [8]. These cytokines have been discovered through the identification of proteins responsible for ectopic bone formation in rodents [9]. They have been studied extensively during the recent decades. Both purified BMPs and recombinant human BMPs (rhBMPs) are widely used in several GTR technics, with the aim of promoting complete regeneration of bone, ligament, and cement [10-12].

BMPs are crucial growth factors in bone formation and healing. They stimulate chemotaxis, proliferation, and differentiation [8]. However, protocols for clinical use are divergent, calling for high doses of BMP, which could result in unwanted side effects such as ectopic bone formation, peri-implant bone resorption [15], swelling [14], and generalized hematomas in soft tissues [16], besides its expensive nature [14]. These disadvantages could be minimized by the development of techniques that increase the half-life of BMPs, so that they are effective at lower doses [13].

Carriers for BMPs have shown to be effective in increasing the stability of the BMPs *in vivo*, thereby aiding in their function of stimulating bone regeneration at lower doses [17]. A variety of biomaterials such as calcium phosphate cement [18], bioactive glass ceramic [19], hydroxyapatite [20], some hydrogels [21], and collagen [22] has been investigated. Once again, achieving consensus among the researchers about the optimal choice has been challenging.

Moreover, there is no consensus about the optimal concentration to be used in each technique. The concentration used in protocols ranges in the order of micrograms for purified BMP and milligrams for recombinant versions (rhBMP). There are also other variables that influence the outcomes, such as study subjects, experimental sites, and conditions for bone regeneration [23]. Animal studies are not optimal to standardize dosages, because the dosage varies based on the animal models used. In rabbits, 0.67 mg/ml is enough for ectopic bone formation [24]. In rats, 0.01–0.1 mg/ml induces ectopic bone formation. Non-human primates need doses in the range of 1–1.5 mg/ml [25]. Thus, a review of clinical studies is necessary to prescribe a reliable protocol, taking into consideration, the most frequently used dosages and types of carriers.

2.1. Delivering BMPs

Absorbable collagen sponge (ACS) was the first BMP-carrier technology to be approved by the United States Food and Drug Administration. It has proven to be a reference standard for techniques of GTR that included a growth factor [26]. ACS can be used alone or in combination with bone substitute materials, such as beta-tricalcium phosphate, biphasic calcium phosphate, and bovine bone mineral [27]. It has characteristics that are important for its carrier function, such as biocompatibility, defined release kinetics, clinical applicability, and uneventful biodegradation, [28].

In addition to ACS, collagen membrane loaded with small doses of rhBMP or BMP also enhanced the two-way vertical bone regeneration [29-

32]. Thus, many studies have shown the advantages of the combination of BMPs and collagen, especially ACS [10, 22, 34].

ACS proved to be a good BMP carrier with important safety background. However, collagen sponge demonstrated some disadvantages owing to its xenogeneic origin [35], which can elicit an immune response. Clinical trials demonstrated that approximately 18% of the patients treated with BMP+ACS developed anti-type I collagen antibodies [35]. Therefore, other biomaterials have also been developed with the objective of improving the efficacy of BMPs, while functioning as an extracellular matrix [17, 36, 37]. Synthetic porous polymers have been shown to be promising biomaterials for this purpose [36]. They facilitate the infiltration of osteogenic cells, and promote cell proliferation during bone formation [38].

Bioabsorbable polymers of lactic and glycolic acid (PLGA) and tricalcium phosphates, and porous beta-tricalcium phosphate (β -TCP) are the most commonly used biodegradable and osteoconductive biomaterials [39, 36]. Although these biomaterials yielded good results, a study comparing ACS with PLGA demonstrated greater bone regeneration when ACS was used [22]. The authors believe that the collagen protein facilitates enhanced cellular ingrowth and/or attachment compared to the synthetic co-polymer and that the difference in the degradation mechanism of the two carriers is responsible for the better results obtained with collagen sponge [22].

A study aimed at improving the technique that linked PLGA-based biomaterials with zirconium oxide or collagen [36]. These polymeric scaffolds with added ceramic particles lacked optimal mechanical properties, whereas calcium phosphate cement embedded in PLGA failed to release the encapsulated therapeutics completely. Despite these limitations, these materials demonstrated good results, indicating that composite scaffolds can improve bone tissue regeneration [36], and that biodegradable polymeric microspheres are ideal vehicles for controlled delivery applications of drugs, peptides, and proteins [39, 36].

Dextran-derived, biodegradable hydrogel microspheres have also been proposed as carriers for BMP [17, 37]. They are an attractive option for

drug delivery because they combine good tissue biocompatibility with the manipulability of their permeability to solutes [17]. The authors concluded that dextran-derived hydrogel microspheres may have an excellent potential, because of their capacity to absorb or get cross-linked to specific ligands or adhesion agents that target the hydrogel microspheres to periodontal tissue or defects [17, 37]. However, the *in vitro* release kinetics indicated that those hydrogels release the BMPs in a rapid burst and could retain BMPs only for a few days, indicating a possible disadvantage of the material [21].

Nanotechnology has also been proposed in the search for ideal conditions that enhance GTR. Membrane-based nanofibers were created [40, 41]. A core-shell structure of a nanofibrous barrier membrane in combination with rhBMP-2 was developed as a sustained delivery device of rhBMP-2 [40]. It has been shown that biologically active BMP-2 can be released over a prolonged period from the core of the coaxial electrospun membranes *in vivo* and *in vitro* [40]. However, this methodology has some disadvantages. Pore size and porosity cannot be controlled; there is large batch-to-batch variation and poor biomechanical strength [42].

From these studies, it can be concluded that ACS impregnated with BMPs or collagen membranes with bound BMPs seem to be the most suitable material for GTR, by stimulating cell proliferation and promoting greater odontoblast and osteoblast action [22]. Although synthetic biomaterials seem to have promising characteristics, investigations into clinical translation are still scarce. Nanotechnology concepts too aid in the development of newer techniques and new materials.

2.2. BMPs in GTR

Techniques employing BMPs in combination with xenogenic bone substitute mineral in clinical settings have been described. There is a report in which 0.5 g of xenogenic bone was uniformly moistened with 1 mL of 0.5 mg/ml rhBMP-2 solution. After 1 h of equilibration, the tubes were placed in sterile lyophilization containers and lyophilized under sterile

conditions. Individual batches were prepared up to 2 weeks ahead of implantation and stored at 4°C until use. The defect sites were grafted with the xenogenic bone substitute mineral with rhBMP-2 (mean dose of rhBMP-2 per patient was 0.18 mg), and the site was covered with a bioresorbable collagen membrane. According to the authors, this protocol increased the graft to bone contact and enhanced the maturation process of new bone [11, 12].

However, the most reliable and clinically used method seems to be the combination of ACS with BMP. This technique has resulted in successful bone regeneration in both preclinical and clinical studies [10, 43]. The inclusion of collagen has improved the technique and currently collagen membranes incorporating growth factors are being studied. A study in dogs used a membrane collagen matrix infused with 0.2 ml solution of rhBMP-2 at a concentration of 0.5 mg/ml [32]. A study in rabbit model used the membrane with 3.85 mg of rhBMP-2 [30]. Another recent study evaluating membranes was performed *in vitro*, in which the membranes were soaked in a solution of 10 ng/mL of rhBMP-2 [31]. Histological observations confirmed that substantial bone regeneration had occurred in rhBMP-2-treated defects. Ankylosis, when present, was generally limited to regions immediately apical to the cemento-enamel junction. The newly formed bone assumes the characteristics of the adjacent resident bone and allows placement, osseointegration, and re-osseointegration, and functional loading of endosseous implants [44].

The combination of BMP + ACS has been widely used clinically in cases of lumbar spine fusion surgery [45]. In a large study reporting consecutive series of posterolateral fusion cases using rhBMP-2/ACS (1036 patients), there were extremely few complications directly attributed to rhBMP-2/ACS. The overall complication rates were consistent with established norms. In this review, it was observed that the amount of rhBMP-2/ACS used was a single large kit (12 mg, 1.5 mg/ml) in 970 cases (93.5%) [45].

In another study, patients treated with rhBMP-2/ACS in extraction sites or in sites that required alveolar ridge augmentation were followed up for 3 years [43]. The technique was successful in most cases. The amount

of rhBMP-2/ACS implanted in patients depended upon the procedure and the defect size. Consequently, the total rhBMP-2/ACS dose per patient varied, although the concentration of the rh-BMP-2 solution used remained the same (0.43 mg/ml) [43].

Animal models do not seem to be ideal for the determination of optimal dose of rhBMP or BMP, because of the large variation in the reported doses. In clinical studies in humans, the average concentration of rhBMP used was 0.43 mg/mL [43]. The results of these studies suggest that this concentration is neither too high to cause undesirable side effects, nor too low to be ineffective. The regeneration observed in the patients was satisfactory.

3. PDGF

PDGF is a heparin-binding polypeptide and has mitogenic, proliferative, and chemotactic effects on connective tissue cells [46]. The original source of PDGF was platelets, but PDGF or PDGF-like peptides have been isolated from a variety of normal and neoplastic tissues, including bone matrix and osteosarcoma cells [47]. PDGF at the wound site originates from the increased number of mesenchymal cells in the wound [48]. This PDGF diffuses into the surrounding tissue and acts as a chemoattractant, recruiting cells into the wound, and increases cell proliferation [49].

Studies have shown that PDGF may be useful for stimulating bone regeneration [50, 51]. This occurs because the bone regeneration begins by the initiation of mitosis in stem cells and endothelial cells, as well as the activation of osteoblasts and vascular growth directed by PDGF [52]. There have been studies investigating the effect of including PDGF in GTR on bone regeneration. This technique has been used in some clinical studies and showed favorable results [53-57]. The discovery of PDGF has opened up new avenues for developing better and novel techniques to treat wounds and also opened up new possibilities to regenerate bone in fracture

areas or to augment bone grafts for better and faster bone regeneration [52].

3.1. PDGF in GTR

Preclinical animal studies using PDGF in GTR have demonstrated that PDGF has a stimulatory influence on bone formation, by enhancing the proliferation of cells [50, 51]. Studies seeking to develop techniques with new biomaterials use the amount of PDGF produced by the body in the presence of innovative therapies, as a parameter. This is because of the direct proportionality between the amount of PDGF and degree of mitosis, cell proliferation, and chemotaxis in bone regeneration [58].

A human clinical trial evaluated the clinical and histological response to recombinant human platelet-derived growth factor (rhPDGF) delivered in bone allograft, in the treatment of advanced class II furcation defects [53]. In this study, four molars with advanced class II furcation defects were selected. After scaling and planing and obtaining all measurements, the root surface was conditioned with a tetracycline paste for 4 minutes for decontaminating and removing the smear layer. Demineralized freeze-dried bone allograft, was saturated with a solution of rhPDGF (0.5 or 1.0 mg/mL), and the rhPDGF/allograft mixture was allowed to sit for about 10 minutes, before implantation. Appropriate post-operative care was provided. After nine months, the region of the original osseous defect and adjacent tooth structure was removed en bloc for histologic analyses. Both clinical analysis and histological analysis showed the formation of autogenous bone, interspersed with collagen fibers over enamel projections within the furcations. There was no significant difference in the outcomes with different dosages of rhPDGF [53].

Another clinical study was conducted with 180 patients with advanced periodontitis, or requiring the extraction of at least one tooth due to extensive interproximal intra-bony and/or molar class II furcation defect. Using PDGF in the treatment of these defects resulted in substantial gain in clinical attachment level and reduction in horizontal probing depth [55].

When the effectiveness of two different doses of PDGF (0.3 and 1.0 mg/ml) in combination with β-tricalcium phosphate (β -TCP) was compared with that of β -TCP alone in deep intra-osseous defects, the rate of gain in clinical attachment was shown to be more rapid in the low-dose PDGF + β-TCP group compared to that in the control group at 3 months post-surgery. However, after 6 months, there was no difference between the two protocols [55].

The same authors conducted other clinical studies [54, 56, 57]. Patients with severe periodontal disease were treated with different matrices mixed with 1.0 mL (0.3 mg/mL) rh-PDGF per gram of xenograft and allowed to absorb for 10 minutes. The graft was fixed with screws and the region was covered with a resorbable membrane. Radiographic [56] tomographic and histological analyses [57] were performed after five months. All the three different kinds of analyses demonstrated an increase in the bone in treated region, showing that the protocol used can be an alternative for cases of severe periodontitis [56, 57].

Based on the published clinical studies, it can be concluded that the inclusion of PDGF in GTR seems to be a great option for bone regeneration. Both protocols, with and without membranes, yielded successful outcomes [53-57]. However, further research is necessary to standardize the technique.

4. PTH

PTH is a polypeptide synthesized and secreted by the parathyroid glands. PTH binds to cells of the osteoblast lineage and produces both anabolic and catabolic effects [59]. PTH has been associated with therapies aimed at bone regeneration, because of its ability to stimulate osteogenesis [60]. The striking clinical benefit of PTH in osteoporosis began a new era of skeletal anabolic agents [61]. It has already been approved by the U.S. Food and Drug Administration for the treatment of osteoporosis in both women and men. Studies investigating the benefit of using PTH along with GTR are in progress, and the results have been promising [62].

4.1. PTH in GTR

The effect of PTH in critically sized rat calvarial bone defects was investigated [62]. A full-thickness bone defect (diameter 5 mm) was trephined under constant saline irrigation. The bone defects were covered with an endocranial polytetrafluoroethylene membrane between the dura mater and the parietal bone and an exocranial membrane was placed between the periosteum and parietal bone. The animals were injected daily for 35 days (the duration of the study), with 60 μg of PTH/kg. The results demonstrated that the treatment with PTH enhances guided bone regeneration and mechanical strength [62].

Another study using dogs demonstrated the local effects of PTH in combination with different matrices, in acute defects around implants, at early post-surgical time points (2 and 4 weeks) [63]. In this study, the bone formation in the defects increased from 2 to 4 weeks in all experimental groups, with or without PTH. After 2 weeks, the presence of PTH marginally improved the bone formation within acute defects created around dental implants [63].

From these studies, it can be concluded that PTH yields satisfactory results *in vivo* in treatments that seek regeneration or increase in bone density, as in the case of osteoporosis. Thus, it is important that new studies reveal the advantages and disadvantages of different protocols for this association to become a clinical reality.

5. FINAL CONSIDERATIONS

Although growth factors have been the subject of much research, especially in the area of tissue engineering, standardization has been difficult, since most of the new materials come with innovative features that complement other materials [40, 41]. Therefore, further studies are needed to establish the effectiveness of growth factors as well as the combination of carrier matrices and growth factors. The clinical effectiveness of the combination of BMP and ACS seems to be well

established [10, 43]. PDGF has also been shown to be clinically effective [53-57]. However, PTH, which shows great promise in bone regeneration procedures, needs to be investigated further [62, 63].

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Chapter 3

THE ROLE OF PLATELET-RICH PLASMA IN GUIDED TISSUE REGENERATION

Mariane M. Azuma*, Luiz C. de O. Rocha, Pedro F. E. Bernabé and Luciano T. A. Cintra
Department of Endodontics, São Paulo State University (Unesp), School of Dentistry, Araçatuba, São Paulo, Brazil

ABSTRACT

Regeneration is defined as the reconstitution of a lost or injured part. This process needs the occurrence of some cellular events during the inflammatory process, such as cell adhesion, migration, proliferation, and differentiation. Combining guided tissue regeneration (GTR) with bone grafting has yielded positive results. The inclusion of platelet-rich plasma (PRP) in GTR has also been studied, but the literature does not give comprehensive information. PRP is defined as a concentrate of platelets in a small volume of plasma and is considered a rich source of autologous growth factors. Among them are platelet-derived growth factor (PDGF), insulin-like growth factor (IGF), transforming growth factor (TGFs/β1

* Corresponding author: Mariane Maffei Azuma, Department of Endodontics, São Paulo State University (Unesp), School of Dentistry, Araçatuba, São Paulo, Brazil, R. José Bonifacio, 1193 Araçatuba - São Paulo, Brazil. Tel.: +(0055) 18 36363253, Fax: +(0055) 18 36363253. Email: mariane_azuma@hotmail.com.

and β2), vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), and the cytokine interleukin-1. The rationale behind adding PRP to bone grafts is that high concentrations of platelets in a bony defect will increase the local concentration of growth factors and subsequently enhance the bone healing response. GTR with PRP has been used for augmenting deficient alveolar ridges prior to or in conjunction with endosseous implant placement, periodontal tissue regeneration, apical surgery, and maxillofacial surgery. There are reports in the literature that showed that PRP promoted bone regeneration. On the other hand, there are also studies that questioned the clinical efficacy of PRP. Thus, the aim of this study was to review the role of PRP in guided tissue regeneration according to published literature.

Keywords: guided tissue regeneration, platelet-rich plasma, dentistry surgery

1. INTRODUCTION

1.1. Guided Tissue Regeneration

Regeneration is defined as the reconstitution of a lost or injured part [1]. Tissue regeneration is a complex process that requires the occurrence of a sequence of cellular events such as cell adhesion, migration, multiplication, and differentiation [2, 3].

Recent literature describes many types of techniques that could be employed to promote tissue regeneration. Most common among them are the use of bone graft materials in conjunction with guided tissue regeneration (GTR) and, more recently, the use of polypeptide growth factors (PGFs) in GTR [3]. Previous studies have shown that combining bone grafting with GTR yielded better results when compared with those of other techniques employing GTR alone [4-7]. It is believed that while a barrier addresses the dynamics of cell migration, osseous grafts play an active role in promoting the formation of alveolar bone [7]. The placement of a physical barrier over an osseous defect may prevent the ingrowth of the faster proliferating oral epithelium and gingival connective tissues into

the bone defect, thus allowing the cells of periodontal ligament and endosteum to colonize the blood clot and regenerate the lost tissue [8].

GTR therapy, introduced in the 1980s, has been widely used to regenerate tissues lost to periodontal disease, such as periodontal ligament and alveolar bone. GTR has also been used in the apical surgeries as a concomitant treatment during the management of endodontic-periodontal lesions [9-12]. In addition, GTR has been used in mandibular defects, defects around implants, and intra-bony defects [13-14].

In addition to the advantages described, GTR has some disadvantages too, such as high cost, difficulty in proper flap approximation, possibility of infection, and a greater risk of mechanical trauma resulting in micro endo-perio communications [15]. Some studies have also demonstrated root resorption and ankylosis with the use of GTR membrane [16, 17]. A membrane barrier may actually prevent osteoprogenitor cells in the periosteum from migrating into the bone defects to aid new bone formation [19].

Studies have shown that platelet-rich plasma (PRP) can be used in combination with GTR [14, 18]. This chapter discusses the role of PRP in GTR.

2. PRP

2.1. PRP Definition and Characteristics

PRP is defined as an autologous concentrate of platelets in a small volume of plasma and is considered to be a rich source of autologous growth factors [20].

Platelets are colorless cell fragments, produced when the cytoplasm of bone marrow cells, termed megakaryocytes, fragments and enters the circulation [21]. Platelets cannot replicate; the life span of a platelet is 5 to 9 days, at which time they are removed from the bloodstream by macrophages [22]. Platelets are approximately 3.0 to 0.5 μm in diameter and are characteristically discoid in shape [23, 24]. They do not have a

nucleus, but contain organelles such as mitochondria and granules. The α granules in platelets contain more than 30 bioactive proteins that play a pivotal role in hemostasis and hard and soft tissue healing [23]. Each platelet has approximately 50 to 80 α granules [23].

The α granules present in the platelets release molecules such as platelet derived growth factor (PDGF), transforming growth factor β (TGF-β), insulin-like growth factor (IGF), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), platelet factor 4, interleukin-1 (IL-1), platelet-derived angiogenesis factor, platelet-derived endothelial growth factor, epithelial cell growth factor, osteocalcin, osteonectin, fibrinogen, vitronectin, fibronectin, and thrombospondin [23, 25-29]. These growth factors, cytokines, and chemokines play key roles during the inflammatory response, promoting chemotaxis, cell proliferation and differentiation and angiogenesis, all of which are essential for tissue repair and regeneration [30]. The main characteristics of these factors are summarized in Table 1.

Table 1. The role of growth factors and cytokines in PRP in bone tissue regeneration

Mediator	Function
Platelet derived growth factor (PDGF)	A chemo attractant, recruiting cells into the wound Increases cell proliferation Regulates the number of cells in the wound and the deposition of matrix Activates cell membrane receptors on target cells Participates in mitogenesis, angiogenesis, and macrophage activation [42-48]
Transforming growth factor β (TGF-β)	Coupling the activities of bone-resorbing osteoclasts and bone-forming osteoblasts Accelerates deposition and maturation of collagen Promotes chemotaxis of fibroblasts Stimulates collagen and fibronectin production Inhibits collagen degradation [49-53]

Insulin-like growth factor (IGF)	Promotes bone cell growth Promotes cell differentiation Promotes cell cycle progression Increases the activity of preexisting bone cells [54-57]
Vascular endothelial growth factor (VEGF)	VEGF is the most powerful angiogenic factor known to date [58]
Epidermal growth factor (EGF)	Promotes endothelial cell migration and endothelial tube formation [59]
Platelet factor 4	Promotes chemotaxis of fibroblasts and monocytes Inhibits collagenase [52]
Interleukin-1 (IL-1)	Activates growth factor expression in macrophages Activates growth factor expression in fibroblasts Activates growth factor expression in keratinocytes [60, 61]
Platelet-derived angiogenesis factor	Promotes mitogenesis of endothelial cells Enhances angiogenesis [52]
Platelet-derived endothelial growth factor	Stimulates mitogenesis of endothelial cells Stimulates mitogenesis of keratinocytes [52
Epithelial cell growth factor	Stimulates endothelial chemotaxis Promotes angiogenesis [51, 52]
Osteocalcin	Participates in the bone tissue mineralization [52]
Fibrinogen	Participates in the primary hemostasis [62]
Vitronectin	Participates in cell adhesion Participates in thrombus formation Participates in mitogenesis Participates in hemostasis [52, 63]
Fibronectin	Participates in cell adhesion Participates in thrombus formation Participates in mitogenesis Participates in hemostasis [52, 63]
Thrombospondin	Participates in cell adhesion Participates in thrombus formation Participates in mitogenesis Participates in hemostasis [52, 63]

According to Mark et al. [31] concentration of platelets at surgical sites increases by up to 338% by the application of PRP. Although there are

gaps in the literature about the beneficial role of PRP in GTR, it is being used quite commonly owing to the ease of clinical application and the possibility of beneficial outcomes, such as faster healing and tissue regeneration [32]. These speculations have led to more studies aimed at a better understanding of the mechanisms by which PRP enhances GTR.

The favorable effects of PRP on tissue regeneration and repair may be attributed to its angiogenic proliferative effects brought about by TGF-β and PDGF. These growth factors present in PRP in high concentrations promote osteoblast differentiation [20]. It has been suggested that using PRP in dentistry may yield beneficial outcomes in tissue regeneration, bone repair, and general wound healing [34]. It has also been suggested that PRP, owing to its high fibrin content, functions as a hemostatic and stabilization agent and therefore aids clot formation and immobilization of the bone substitutes [3, 35]. In addition, PRP has the potential to act as a barrier membrane in GTR and prevent apical migration of the epithelium [36]. Autogenous PRP does not carry the risk of disease transmission or triggering immune response [36]. Using PRP alone is still controversial [37, 38] and therefore combining PRP with bone graft materials such as autogenous bone [39], allografts [40], and alloplastic bone grafts [41] as well as membranes in GTR [3, 18, 36] is the preferred method for regenerating lost tissue. There are a few reports in the literature on the use of PRP in combination with GTR in dentistry.

2.2. PRP Preparation Methodology

PRP can be prepared in a laboratory or an operating or dental room from patient blood collected immediately before surgery. The small amounts of autologous PRP required for dental procedures can be prepared in minutes and involves very little effort. PRP is essentially a concentrate of autologous platelets prepared by centrifugation and suspended in a small amount of plasma. Several systems and instruments have been developed to isolate PRP, all with varying platelet and growth factor yields [64, 65].

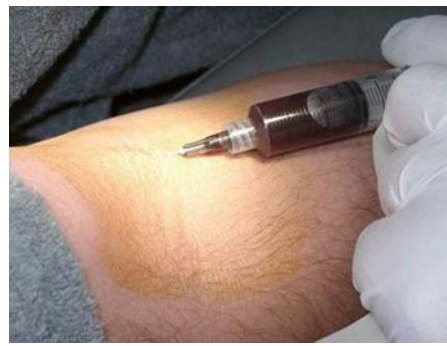

Figure 1. A small volume of the patient's blood is obtained (about 20 ml).

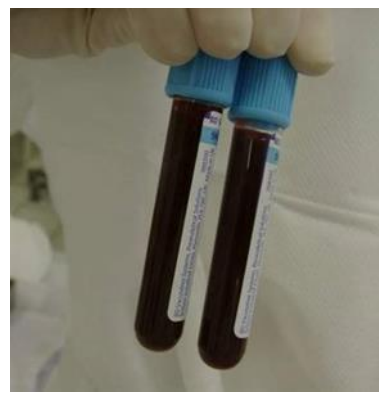

Figure 2. This blood is placed in two tests tubes containing anticoagulant.

Figure 3. The tubes are centrifuged for ten minutes at 800 rpm, which separates the blood into 3 layers: platelet-poor plasma, platelet-rich plasma, and red blood cells.

3. USE OF PRP AND GTR IN DENTISTRY

3.1. GTR in Combination with PRP in Periodontal Defects

Regeneration of periodontal defects involves the reconstruction of alveolar bone, periodontal ligament, and cementum to their original levels before they were damaged by the progression of periodontal disease [66]. Currently, it is possible to obtain positive results with periodontal therapy. However, complete regeneration of bone tissue, periodontal ligament, and cementum tissue does not occur in some cases [67].

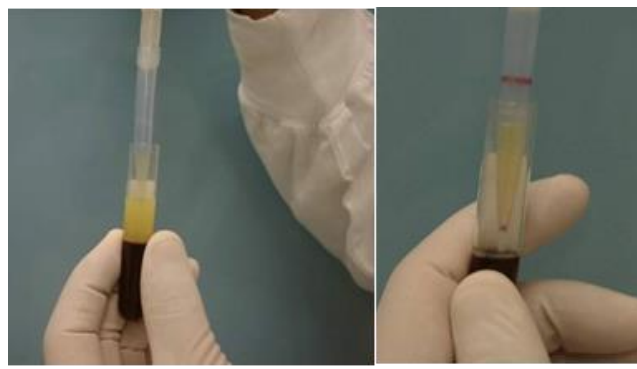

Figure 4. The layer (PRP) immediately above the erythrocyte layer is pipetted out and placed in a test tube.

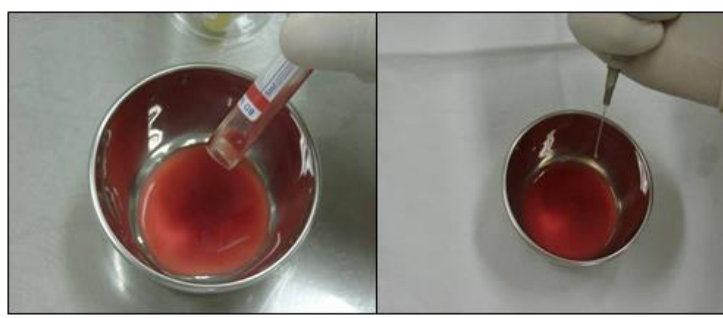

Figure 5. The separated PRP is mixed in a sterile trihydrate metal container with 3 drops of 3.3% calcium chloride.

Figure 6. It is allowed to set into a gel (coagulation).

There are some human clinical trial reports describing the beneficial effect of PRP on bone formation in bone augmentation procedures [26, 68-70]. In the last few years, PRP, combined with different types of grafting materials and barrier membranes, has also been used in regenerative periodontal therapy [3, 36, 71-74].

One case series [75] and one histologic study [76] comparing the autologous platelet concentrate with a bioabsorbable membrane in periodontal defects found similar results in both the groups, suggesting that the autologous platelet concentrate could be used in lieu of a membrane for periodontal GTR applications [75, 76].

Camargo et al. [3] evaluated the effect of PRP in combination with a bone allograft in GTR. Eighteen patients participated in this study. Using a split mouth design, interproximal bony defects were surgically treated with either an absorbable membrane made of polylactic acid for GTR or a combination of PRP, bovine porous bone mineral (BPBM), and GTR. Changes in pocket depth, attachment level, and defect fill as revealed by 6-month reentry surgeries were evaluated. The results of that study suggest that PRP and BPBM provide an added regenerative effect to GTR in promoting the clinical resolution of intra-bony defects in patients with severe periodontitis.

Lekovic et al. [36] reported that the PRP/BPBM/GTR combination technique is an effective regenerative treatment modality for mandibular grade II furcation defects. Further studies are necessary to elucidate the

role played by each of the components of the combined therapy in achieving these results.

Camargo et al. [77] concluded that PRP does not enhance the bone regeneration in intra-bony defects treated with BPBM/GTR combination. It must be emphasized that this study used a relatively small sample size. Hypothetically, if similar studies with a larger sample size (>61 paired defects) were performed and the results were the same as in the report they published, the differences between the experimental and control groups would be statistically significant. However, even in that hypothetical scenario, the clinical benefits of adding PRP to BPBM and GTR would be marginal.

Demir et al. [78] describes a case report in which a deliberate replantation was performed with PRP, bioactive glass graft material, and non-resorbable PTFE membrane. The case was followed up for one year. They concluded that intentional replantation may be used for the teeth, which have no alternative to extraction. Although it is very difficult to make a conclusion with only one case, the authors suggested that combination regenerative techniques may be used in intentional replantation in order to have better and more predictable results.

Dori et al. [79] performed a study with the objective of understanding the extent to which the use of PRP may enhance the clinical outcome compared with treatment with natural bone mineral + GTR treatment. The following clinical parameters were evaluated at baseline and at 1 year after treatment: plaque index, gingival index, bleeding on probing, probing depth, gingival recession, and clinical attachment level. Clinical attachment level changes were used as the primary outcome variable. The results showed that within its limits, the results of the study at one year after the regenerative surgery demonstrated that both treatment modalities of using natural bone mineral + PRP + GTR and natural bone mineral + GTR, yielded significant reduction in probing depth and increase in clinical attachment level. They concluded that PRP failed to improve the results obtained with natural bone mineral + GTR.

3.2. GTR Combined with PRP in Apical Surgery

The PRP and GTR combination technique may be used in apical surgery as described by Goyal et al. [18]. In this study, thirty patients with suppurative chronic apical periodontitis and apico-marginal communication were selected and allocated randomly into three groups according to the barrier technique to be used during periradicular surgery: the collagen membrane group, the PRP group, and the PRP + collagen sponge group. Clinical and radiographic measurements were done at the baseline and every 3 months after surgery up to 1 year. Cases were defined as healed when no clinical signs or symptoms were present, and radiographs showed complete or incomplete (scar tissue) healing of previous radiolucencies. The results showed that PRP and PRP + collagen sponge groups exhibited 83.33% and 88.89% healing, respectively, in terms of combined clinical-radiographic parameters as compared with the 80% in the collagen membrane group. All the three treatments showed highly significant reduction in the periodontal pocket depth, increase in clinical attachment level, improved gingival margin position, reduction in the size of periapical lesion, increase in the percentage reduction of the periapical rarefactions, and improved periapical healing. No significant differences between the three groups were evident with respect to these parameters. The authors concluded that GTR applied to apicomarginal defects using PRP or PRP + collagen sponge resulted in similar clinical outcomes of periradicular surgery in terms of periapical healing, gain of periodontal support, and PD reduction, and that PRP may be an alternative to GTR in the treatment of apicomarginal defects.

No other studies evaluating the use of PRP as an aid to healing of apical defects have been reported in the literature.

3.3. GTR Combined with PRP in Intra-Bony Defects

PRP was first introduced in 1998 to treat mandibular intra-bony defects [26]. When autogenous PRP was combined with bone grafts for

reconstructing mandibular bone defects resulting from the removal of tumors, the resulting bone density and maturation rate were higher [80]. Since then, PRP has been used in periodontal and maxillofacial surgery [39], as well as aesthetic plastic surgery [81] to improve the healing of maxillofacial bony defects.

De Vasconcelos et al. [82] histometrically evaluated bone healing in surgically created dehiscence-type defects around titanium implants, treated with GTR in combination with PRP and concluded that PRP does not enhance the bone regeneration effect of GTR.

Sammartino et al. [14] showed that using PRP in association with absorbable collagen membrane of porcine origin (Bio-Gide; Geistlich Biomaterials, Wolhusen, Switzerland) for the prevention of periodontal complications that may follow the extraction of deeply impacted lower third molar, yields only a limited increase in bone regeneration when compared to PRP alone. The authors conclude that this difference cannot be considered to be clinically relevant. The combined use of PRP with absorbable membrane seems to favor only a faster maturation of the regenerated bone in the surgical site.

Fu and Wang [83] conducted a meta-analysis and concluded that PRP was beneficial in the treatment of intra-bony defects when used with graft materials but not with GTR. According to the authors, the use of PRP did not enhance soft tissue healing and regeneration in the treatment of gingival recession.

Chen et al. [80] evaluated, histologically and radiographically, the effect of autologous PRP when combined with BPBM and bioguide membrane (BGM) in promoting mandible bicortical bony defects in rabbits. The study suggested that the addition of PRP to BPBM and BGM had significant therapeutic benefits in mandible bicortical bony defect regeneration in rabbits. The effects were attributed to the high concentration of platelets in PRP and the porous configuration of BPBM. Although we are not able to present the statistical significance of the effect of adding PRP to the osteoinductive effects of BPBM/GBM, we concluded that PRP demonstrated good results of bone regeneration.

4. FINAL CONSIDERATIONS

According to the current literature, using PRP in GTR yielded results similar to those obtained with GTR alone. PRP may be used as an auxiliary to GTR to treat bony defects by surgery, in the light of some positive clinical results obtained when PRP was used as a complementary treatment for bony defects.

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Chapter 4

GUIDED TISSUE REGENERATION IN APICAL MICROSURGERY

Luciana L. Ferreira, PhD, Pedro F. E. Bernabé, PhD and Luciano T. A. Cintra, PhD
Department of Endodontics, São Paulo State University (Unesp), School of Dentistry, Araçatuba, SP, BR

ABSTRACT

Endodontic treatment aims to eliminate bacterial infection of root canal. However, the infection persists in some cases even after endodontic treatment. Periradicular surgery is an established treatment option in endodontics. Guided tissue regeneration (GTR) has been proposed in conjunction with apical surgery in order to obtain better bone healing. The fundamental principle of this technique is the prevention of the invasion of the bony defect by faster replication of junctional and gingival epithelial cells with the help of a barrier membrane placed over the material grafted in the defect. This protection facilitates the proliferation of cells of the periodontal ligament and alveolar bone in the defect, leading to the regeneration of bony defects. Techniques have been developed to enhance the GTR. However, the use of GTR technique should not become a routine procedure, since the periosteum is itself a barrier to epithelial and connective tissue cells. With respect to the

membranes used in GTR, the literature has shown some preference to resorbable membranes and natural bone mineral for intra-bony defect filling. The aim of this review is to evaluate and compare the results of various studies that used the GTR, advantages and disadvantages of GTR, and the use of membranes and grafts.

Keywords: GTR, membranes, grafts, apical surgery

1. INTRODUCTION

1.1. Apical Surgery

In endodontic treatment, the terms apical surgery and apical microsurgery indicate the same procedure. This procedure is indicated when conventional endodontic treatment or retreatment has failed. The main objective is to create a healthy environment for apical tissue healing. It involves the removal of pathological tissues and cortical bone to gain access to the periapical area and then a primary closure of the surgical site with healthy tissue [1].

As with any other surgical procedure, apical microsurgery is indicated in the following situations as proposed by the Spanish Society of Oral Surgery and the European Society of Endodontology: I) periapical disease affecting a permanent tooth subjected to a good endodontic treatment, with pain and inflammation; II) periapical pathology with prosthodontics or conservative restoration that proved to be difficult to remove; III) a radiolucent lesion measuring over 8 to 10 mm in diameter; IV) Symptomatic gutta-percha overfilling, or presence of a foreign body not amenable to orthograde removal (fractured file); V) perforation of the root or the floor of the pulp chamber.

The non-indications for apical microsurgery are: the tooth has no function, the tooth cannot be restored, has compromised periodontal support or root has vertical fracture. In addition, if the patient is uncooperative or with compromised health, the surgery is non-indicated [2].

1.2. Classification of Apical or Endodontic-Periodontic Lesions

In theory, any aggression to the endodontic or periodontic systems can cause a reaction on the other. In most cases, it is easy to distinguish between endodontic and periodontic lesions, but sometimes the diagnosis can be difficult and doubtful [3]. Simon proposed an endodontic-periodontal lesion classification based on its etiology [4]:

1. Endodontic lesions: Clinical examination can show some dental mobility, loss of bone in furcation or crest, percussion sensibility, gingival sulcus or gingival crevicular fluid, malodour and gingival tumefation. At first glance, the primary endodontic lesions are of periodontal origin. The path can be probed with a gutta-percha cone or with a periodontal probe toward the source of irritation, usually an apex or lateral canal. The determination of pulpal sensibility is necessary to accurately diagnose these lesions, which can be asymptomatic. Once the problem is determined to be of endodontic origin, with periodontal ligament fistula, its complete resolution is sometimes just after conventional endodontic treatment.
2. Endodontic lesions with secondary periodontal involvement: If the endodontic disease is not treated, secondary periodontal disease can develop. As the crevicular fluid persists through the gingival sulcus, the plaque and calculus result in a periodontal pocket and apical migration of supporting structures. Generally, the endodontic healing occurs first.
3. Periodontal lesions: They are caused by periodontal disease. Periodontitis progresses up the entire root surface to the apex. Occlusal trauma may or may not be involved in the development of these lesions. Periodontal probe reveals the presence of calculus on an extended area of the root surface and the pulp tissue reacts positively to the pulpal tests. The diagnosis must include a radiographic examination and a cavity test may be necessary. The pulp vitality suggests that favorable prognosis depends on the

periodontal therapy. Gingivitis and initial periodontitis can be treated; if not treated, deep periodontal pockets can develop with continuous loss of insertion, necessitating surgical procedures.

4. Periodontal lesions with secondary endodontic involvement: This situation occurs due to progression of the periodontal lesions up to the apex, or to the accessory root canals or laterally, exposing the pulp to the oral environment, leading to pulp necrosis. The tooth presents, in these circumstances, deep periodontal pockets indicating a history of extended periodontal disease. Pulp necrosis can result from periodontal procedures, when the blood supply might be interrupted or inefficient [5]. The prognosis depends on continuity of periodontal treatment after endodontic therapy.
5. Endodontic-periodontic lesions: The progression of apical lesions with epithelium migration of advanced chronic periodontal pocket can be encountered at any position along the root. Independent pathological processes can converge to form a combined lesion, and simulate an endodontic lesion. The repair of endodontic aspect occurs after the endodontic treatment, but the periodontal repair may or may not respond to the periodontal treatment, depending on the severity of involvement.

There is a dynamic relationship between pulp and periodontal tissues; they have the same embryonic origin and have an intimal anatomic and functional relation [6]. Branching of the pulpal cavity, dental fractures, development of cracks and grooves in the crowns and roots are the main factors responsible for the changes in the products and subproducts among these tissues [6-10].

Another important relationship between these tissues is the similarity of microorganisms present in infected root canals and lateral periodontium of the lesions. A study revealed that strict anaerobic bacteria dominate over 90% of the microbiota of infected root canals. The microbiota of a root canal is not as complex as that in the periodontal pocket [11]. According to Whyman, deep periodontal pockets normally expose the dentinal tubules after the removal of necrotic cementum during periodontal therapy [12].

This exposure leads to pulp contamination through lateral periodontium. Bacteria and their products cause pulp inflammation, compromising the blood vessels and tissue nutrition, resulting in pulp necrosis [5].

The diagnosis of pathological alterations in pulp and periodontium is important for deciding appropriate therapy aimed at restoring the lost structures of endodontic and periodontic tissues and for the success of that treatment [9].

Undoubtedly, the endodontic-periodontic lesions have worse prognosis, and many times, the option available to the dentist would be extraction [13]. However, treating these lesions with GTR can result in a favorable prognosis [14].

von Arx and Cochran proposed in 2001, a lesion classification for membrane application in apical surgery. Class Ia lesions showed bony defects at the apex without marginal lesion, and Class Ib lesions included through-and through bone defects [15].

1.3. GTR in Apical Microsurgery

The literature on GTR in periapical surgery is quite extensive. This procedure involves the use of biologic and engineering sciences to develop biologic substitutes that maintain, restore, and enhance the tissue function [16]. The use of membrane barriers and/or bone graft in apical microsurgery is an example of tissue engineering [17]. The first report of GTR in dentistry was from Bjorn. He proposed the utilization of some kind of barrier in order to guide the desired cells into the defect for better regeneration of lost tissues. This concept was based on the idea that the epithelial cells are capable of migrating and proliferating faster than bone tissue. Therefore, a barrier has to be used to prevent the migration of epithelial cells and allow the colonization of the defect by cells of the original tissue [18]. Loe and Warehaug showed the importance of periodontal ligament in repair procedures [19].

Melcher [20], Carranza and Kanney [21], Oliveira et al. [22], and Gagnon and Morand [23, 24] reported that the repair or regeneration will depend on the cell type that first fills the exposed root surface.

These reports prompted research into means of slowing down or interfering with the epithelial migration up to apical root, allowing enough time for the connective cells of periodontal ligament to repopulate the root surface and the attachment to get established. One method is to place a membrane or mechanical barrier in contact with bone tissue and cover the barrier with periosteum [25-27]. The objective of placing the barrier is to exclude undesirable cells, giving an opportunity to mesenchymal stem cells to differentiate into fibroblasts, cementoblasts, and osteoblasts, leading to the complete repair of the defects. This treatment method is called guided tissue regeneration (GTR) [28]. Retrospective studies have shown that the success of apical surgery was not as good as expected [29, 30]. Approximately a quarter of the cases were unsuccessful during the decades of 60s and 70s [31, 32]. In the 80s, 50% of the cases were considered successful achieving complete repair, with another 25% showing reduction in the degree of apical periodontitis [33]. If we include other associated conditions such as cortical bone loss or the presence of endodontic-periodontal lesions, these percentages are smaller [31]. GTR is a technique that provides ideal conditions for the complete restoration of original architecture of the lost tissue [23]. The main goal for using GTR in apical microsurgery is to enhance the quantity and quality of the new bone, as well as to accelerate bone growth around the bony cavity after surgery [14].

The reasons for using GTR in apical microsurgery include acceleration of periapical healing and the presence of other clinical conditions, such as large endodontic-periodontal lesions [34, 35]. The high failure rates of apical surgery are directly linked to a variety of factors that influence the repair process of the periapical region [36].

The search for satisfactory results prompted more studies in the area of repair and regeneration of damaged or lost tissues, particularly for those that provide protection to the dental periapex. The purpose was to develop regenerative and reparative techniques that could promote the restoration

of structures that were lost in their entirety, but preserve the biological tissue space [37].

Nyman et al. conducted three studies. They used a membrane in the first study with the aim of achieving complete repair. The conclusion from these studies was the possibility of partial repair of tissues when a barrier of Millipore membrane was interposed between the gingival tissue and exposed root surface and the adjacent bone. The Millipore filter acts as a barrier, preventing the colonization of exposed root surface by gingival cells and allowing the selective repopulation of this surface by periodontal tissue [25-27].

The healing process after the apical microsurgery may be observed by a complete tissue repair with recovery of destroyed structures; a fibrous or scar tissue with many levels of inflammation or by the absence of repairing tissue with mild to severe apical inflammation [14, 23, 24].

Nyman and Gottlow studied the repair process of periodontal tissues in monkeys, searching for a membrane that provides good results using the GTR technique [25-27, 38].

In 1986, GTR was recognized as a technique that reestablishes the contact between connective tissue and root surface that was deprived of periodontal ligament [39]. This contact occurs by the formation of new cement with insertion fibers [40].

According to Melloning and Bowers, there are two important factors in the repair of pathological exposed roots: the use of an osteogenic material to improve the formation of bone, cementum, and periodontal ligament, and the exclusion of fibrous tissues that try to invade the same space as regenerating tissues [41].

The use of membrane in apical microsurgery has the unique function of speeding up the healing and guiding the formation of new bone at the defect site created for surgical access or because of the periapical lesion that existed. In cases of the simultaneous presence of endodontic and periodontal lesions, the membrane has a double function, to establish attachment anew, as well as to guide the bone formation in the bone cavity and on the exposed root surfaces. This membrane does not replace the periosteum, which in itself is a barrier to soft tissue cells. The objective is

to act together and with the same goal; promoting cell selectivity and simultaneously creating the necessary space for the regeneration of tissues. This space created by the presence of the membrane ensures that mesenchymal cells can migrate into the area of repair and differentiate, resulting in osteogenesis without competition from other types of cells [42]. Thus, the contact of the blood clot and subsequent granulation tissue with root surfaces is essential to allow the apical migration of the junctional epithelium and the maturation of connective tissue. This tent-shaped space, created by membranes to support the clot, provides a framework for cellular ingrowth [43-45]. The unique combination of advantages of using membranes leads to the restoration of the architecture of the lost dental tissues.

Some authors used membranes in cases of radicular perforation, because the bone loss caused by creating surgical access or by perforation must be restored [46, 47]. Others applied the technique to large bone defects [48, 49], or in apical microsurgery. Gagnon and Morand used membranes, because membrane use is indicated in cases of nasal cavity or maxillary sinus communication. All authors support the use of membranes when both endodontic and periodontal lesions are present [23, 24].

Douthitt et al. conducted the first histological analysis on the resorbable membranes used in the case of endodontic-periodontal lesions. The authors concluded that resorbable membranes increase the bone growth in periapical zone, facilitate the repair of connective tissue and the growth of alveolar bone upon exposed root surface. Better long-term results were obtained with the use of membranes [30].

The deposition of hard tissue on the root apex and retrofilling material, forms a biological seal resulting in the formation of the periodontal ligament. This by itself does not guarantee success in endodontic surgical therapy. New bone formation involving the periodontal ligament in both the periapical region and the defects of the alveolar crest, is essential to ensure the protection of the new apex.

After the development of membranes or barriers, repair and regeneration have improved, because membranes cause the regeneration process to follow the specifications of the type of tissue required [50].

Thus, we can say that the process that occurs after surgical procedures without the use of membranes is the formation of a junctional epithelium that invades the area intended for the connective tissue attachment, hindering the bone formation on the root surface, and failing to produce the original tissue. Partial new bone formation can occur in such cases, but not the formation of a periodontal ligament that resembles the pre-existing ligament.

When making use of membranes, root isolation, in addition to creating a tent space, allows the healing process to occur by the repair of periodontal structures with total or partial formation of new bone, new cementum, and new periodontal ligament. These structures afford protection to the exposed root surface and return the original architecture to these tissues [50].

The repopulation by cells that previously occupied these locations and restoration of the original architecture of these tissues can be called regeneration [14, 22-27, 30, 48, 51, 52]. However, microscopically, the regeneration process occurs through the proliferation of residual tissue at the bases of the lesion. Such a process, which depends on the differentiation of cells of granulation tissue, is a characteristic of repair process and not regeneration.

The results obtained using the technique of GTR, even in situations of extreme complexity, are significantly better than those obtained without using a membrane. However, using membranes requires special care, especially with regard to the principles on which this technique is based [53]. Thus, GTR is an adjunct to surgical endodontic treatment, especially for tissues that support the dental element, thus affording protection to this area.

Although many studies involve screening for a biomaterial that addresses the requirements of GTR, the principles of the technique are more important than the materials [49].

2. BIOLOGIC PRINCIPLES OF THE TECHNIQUE

The main principle that forms the basis for GTR is cellular selectivity. A barrier is placed upon a bone defect, which might have been filled with bone graft, to hinder the penetration of epithelial and connective tissue cells. This protection affords the time required for periodontal ligament and alveolar bone cells to differentiate, proliferate, and migrate into the bone defect, promoting tissue repair.

This process of repair is a complex and integrated sequence of events that are triggered by disease. The healing of injured tissues needs a synchronized action of many sorts of specialized cells to restore the structural and functional integrity of lost tissues.

The process of repair can be divided into 4 distinct phases: I) cellular proliferation, II) development of connective tissue, III) maturation of connective tissue, IV) bone differentiation or maturation. It is important to know that none of these phases occurs separately. The repair begins from the bottom and sides of the bone defect, exhibiting a centric direction. The main factor that disturbs the repair process is the difference in the rates of formation of epithelial, connective, and bone cells. The rate of proliferation of soft tissues is higher than that of hard tissues, which favors a quick fill of the bone cavity by these cells.

In attempts to stop or reduce the undesirable cell proliferation and penetration into the defect size, a physical barrier was used to separate the bone cavity and the periosteum, thus facilitating the formation of original cells. The barrier limits the growth of soft tissues and allows the occupation of the cavity by bone tissue [14].

The simultaneous presence of periodontal and endodontic lesions calls for combination treatment [13, 14, 23, 24, 53]. The use of membranes fulfills some requirements: guiding the ideal cells into the defect and creating the space needed for the regeneration of alveolar bone crest, connective tissue insertion, and junctional epithelium [50].

When the goal of the treatment is tissue repair or regeneration [22, 48, 52, 54, 55], the aim is to determine ideal conditions for the original cells to

repopulate the area and revert to the original form, structure, and function [14].

In apical surgery, the deposition of cementary tissue over the apex and retrograde obturation material on biological sealing, and the neoformation of periodontal ligament would be the indicators of success.

3. TERMINOLOGY

It is necessary to know about the terms used when referring to GTR, prior to understanding the studies, as well as to differentiate between clinical and histological terms.

3.1. Reattachment

Reattachment is used to describe the new attachment of periodontal ligament connective tissue with the remaining cells of root surface. This situation occurs after an incision and suture of healthy tissues, allowing the fibers to reattach. The new attachment occurs when there is a formation of cementum and insertion of collagen fibers to the radicular surface, in the place of the original periodontal ligament.

3.2. Regeneration

Regeneration is the process of reproduction or reconstitution of lost or damaged tissue through formation of new tissue identical in form, structure, and function to the original tissues. Microscopically, the events that characterize the regeneration process occur because of the residual tissue, where the nearby cells proliferate and reconstruct the lost area. Bone tissue is unable to regenerate because it cannot reconstitute itself from the nearby cells. Therefore, this tissue gets regenerated through the process of repair by cell differentiation of the granulation tissue.

3.3. Repair

Repair is the process of healing through tissues not native to the site. The repair process occurs at the expense of granulation tissue and not of residual tissue. The granulation tissue reconstitutes the lost area by cell differentiation. The result of both the processes is to restore normal function. Thus, regeneration is not the only process that restores function.

Therefore, all the events that occur in a healing process from the periapical tissues must be called repair and not regeneration. The process of regeneration is considered by some as superior to repair, while in fact, this differentiation should not exist. Microscopically, these processes are different, but one is not superior to the other. They are only different and distinct mechanisms of healing. Both regeneration and repair depend on the nature of the lesion, availability of progenitor/stem cells, growth/ differentiation factors, and microenvironmental cues, such as adhesion molecules, extracellular matrix, and associated noncollagenous proteins [17]. Once the concepts are clear, it can be concluded that repair and regeneration are processes that primarily aim to reconstitute and restore lost structures via different mechanisms.

4. GTR IN APICAL MICROSURGERY

4.1. Membranes or Barriers

With the introduction of GTR using membranes, a new treatment modality has evolved, which has been shown to be superior to the technique involving mucoperiosteal flap over the buccally exposed roots [15]. Membranes have been successfully used in different surgical techniques to enhance new tissue formation in the defects created by the lesion or by the surgical technique [1, 56, 57]. Membranes promote the cellular selection and exclude epithelial cells from the defect site and connective tissue from a periodontal wound [1].

The use of membranes is indicated in some cases of apical microsurgery [13, 23, 24, 47, 49, 58], such as in large bone defects, when lesions are spread over two or more teeth or in periapical lesions with buccal, or palatal/lingual wall involvement, in endodontic-periodontal lesions with a communication between the periapical lesion and the alveolar bone crest; in cases of periapical lesions with interproximal bone loss or perforations; and in periapical lesions with maxillary sinus or nasal fossae communication. It can also be indicated in cases of implants or with bone graft.

However, GTR has also been contraindicated in some cases according to some studies [22-24, 48]. The contraindications can be due to local or general factors. The local factors include small lesions with periodontal involvement, presence of vertical root fracture, excessive bleeding, situations that do not permit membrane stability, and the main factor of failure, local infection. The general factors include those that delay the repair process as a whole. GTR is contraindicated for smokers, since tobacco may favor or initiate a local inflammatory process that interferes with repair process. Consumption of alcohol may have a systemic effect, which can influence the repair process. Any other systemic disorder that alters the immunologic system, and damages the normal course of healing, has a negative impact on the success of the technique. The healing disturbs and the uncontrolled biofilm closes the factors that influence the GTR indications.

Various types of physical barriers have been proposed for use in GTR and these fall into two categories: nonresorbable and resorbable.

4.2. Nonresorbable Membranes

The nonresorbable membranes, also called expanded polytetrafluoroethylene (e-PTFE), are biocompatible and highly effective in maintaining space and inducing new bone formation [1]. It was the first material to be investigated for a biologic barrier. Some authors have described as the disadvantages of using the nonresorbable membranes,

specifically the time taken for performing the surgery and the need of a second intervention to remove the membrane [26, 27, 53, 59, 60].

One of the advantages of nonresorbable membranes is the ability to control the length of time that the membrane needs to be in place, which depends on two factors; the size of the bone defect and the rate of new bone formation. The authors suggest that the membrane be removed 6 to 8 weeks after the surgery, assuming that the bone regeneration is complete [16, 61, 62]. Another determinant of the time at which the membrane can be removed is the radiographic result. These membranes do not get vascularized, which facilitates the visualization by radiography. The membrane does not lose mechanical strength over time, allowing prompt removal in case of complications [22].

Nonresorbable membranes can be divided according to the constituent biomaterial and distinct characteristics. Teflon membrane was the first material proposed for a barrier membrane [25-27] with the use of Millipore filters. This material was well accepted by biological tissues. However, it has been shown that these membranes were not effective as barriers when compared to other barriers [63]. Teflon membranes gave way to the development of the expanded polytetrafluoroethylene (e-PTFE) membrane, widely used nowadays.

e-PTFE is biocompatible and inert to tissues, because they do not produce an inflammatory reaction. The major representative of this group is Gore-Tex® membrane. This membrane is one of the most commonly used barrier because of its excellent biocompatibility and clinical success. The membrane is made from polytetrafluoroethylene polymer by a biotechnological process. Its molecular structure gives its main properties, and the low surface energy allows the inner contact with cells. The new Gore-Tex® membrane is composed of a longitudinal woof of PTFE covered by thinner fibers parallel to each other.

There are other membranes of e-PTFE such as TefGen FD®, which is similar to GoreTex®, flexible and resistant to bacteria, and the TefGen Plus®, which increases the area of tissue-barrier contact, which were used in repair processes with high rates of success.

Some studies report the use of latex membranes in GTR procedures [64, 65]. This has not proved to be a good option because they are non-sterile and difficult to sterilize.

Cellulose membranes, made of cellulose microfibrils, are biocompatible, inert, non-allergic, nonresorbable, and available in different shapes and sizes [66]. It was developed from Biofill (used as artificial skin in 1989), and called as Gengiflex®. Sonohara and Greghi demonstrated the capacity of these membranes to stay inert on the subcutaneous tissue of rat. Millipore and Teflon membranes were also tested in the same study. Gengiflex® showed better results [67].

The membrane composed of aluminum oxide, the Alumina, is a biocompatible membrane that possesses some flexibility, which enables its perfect adaptation over the bone tissue [68].

Some membranes are titanium-reinforced, which allow the creation of the space necessary for tissue regeneration. GTAM and Gore-Tex with titanium reinforcement are some examples of these membranes.

4.3. Resorbable Membranes

Resorbable membranes, developed after the nonresorbable membranes, have demonstrated results comparable to those with e-PTFE membrane in animal [30] as well as human [69, 70] studies. Using resorbable membranes obviates the need for a second surgery. They do not interfere in the repair process and reduce the risks of infection [22]. The connective tissue in contact with the membrane degrades the barrier by enzymatic activity or hydrolysis. The inflammatory response is minimal, reversible, and does not interfere in the repair process.

The ideal material to constitute a membrane would be the one that gets resorbed after the bony defect gets filled with osteogenic cells. If the rate of membrane degradation is higher than that of new bone formation, complete repair will not occur, because the undesirable cells will invade the bone cavity [13].

The membranes most commonly used are those made of collagen, polyglycolic acid, polylactic acid copolymers [34] and other materials, such as polyurethane, acellular dermal matrix, dura mater, chitosan, periosteum, and calcium sulfate [1].

Collagen was one of the first materials used as a barrier. It plays an active role in wound healing process increasing the rate of migration of coronal progenitor cells of periodontal ligament onto the root surface. Some studies have reported better results when the collagen membrane (CM) was used in conjunction with bone graft or collagen gel [71-73,75]. Collagen is resorbed via phagocytosis and the rate of resorption is influenced by local microenvironment, such as area vascularity, mucosa thickness, inflammation, tissue pH, infection risk and partial exposure to buccal environment [76]. The bioabsorption of CM and its impermeability to cell passage are well proven and confirmed [77, 78].

Collagen can be obtained from either human or bovine tissues. Some immunologic response can be triggered by collagen fibers in a process of immunologic cross reaction [79-81]. The first study about human collagen was by Busschop and Boever in 1983. They used the lyophilized human duramater as a repair membrane in the treatment of interproximal defects. They observed that membrane resorption started at 2 weeks and got completed at 6 weeks [82]. Alloderm® is a barrier membrane processed from cadaver skin. The components of this membrane help the cellular repopulation process. The collagen, elastin, proteoglycans, and the blood vessels in the cadaver skin are preserved during processing. These components contribute to the repair process. Several studies showed the effectiveness of this membrane in repair processes [83-86]. Bovine collagen membrane is highly biocompatible and does not elicit significant inflammatory or immunologic reaction [71, 87-89]. Bovine collagen membranes are produced from type I collagen present in joints and tendon. Its resorption takes 4 to 7 weeks, but for bigger bone defects, membranes with extended resorption period are available, namely BioMend Extend® and Bio Gide®, which last for 18 weeks and 16 to 24 weeks, respectively. It is difficult to compare these membranes to any experimental models because of their high density. A study tested the effectiveness of these

membranes in 12 patients and observed that bovine membranes are capable of promoting complete repair [90].

Among polylactic acid membranes, Guidor® stands out. This membrane was studied and compared histologically and histometrically with Millipore filters. Guidor® showed better results with new insertion of 46% against that of 26% with Millipore filters [25-27, 45]. The Guidor® membranes have been studied further, and yielded better results compared to those with other membranes [80, 91-93]. Another membrane is Atrisorb® that seems to meet the clinical expectations.

A new absorbable membrane, called Resolut XT® is composed of a porous synthetic bioabsorbable glycolide and trimethylene carbonate copolymer fiber and is an occlusive membrane of synthetic bioabsorbable glycolide and lactide copolymer. This membrane was designed to be stiff enough to create and maintain a protected defect space in order to establish an environment favorable for regeneration, yet supple enough to drape smoothly over the defect margin. Its complex structure affords it a life of 8 to 10 weeks at the defect site.

Vicryl® (Polyglactin 9.0), prepared from glycolide and lactide copolymer is the main representative of polylactic acid membrane group. It is totally absorbable, inert, non-allergic, and is absorbed with a minimum tissue reaction. The advantages of this membrane are the low cost, ease of use, and availability in different shapes and sizes. Some studies have demonstrated that its use permits a new connective insertion on roots exposed by periodontal disease, both in dogs [19, 45] and humans [28, 94], and allows good osteogenesis and cementogenesis [95].

Despite the advantages of membranes in tissue healing, we must also consider the possibility of complications such as flap necrosis, usually caused by cell death or poor blood circulation. Periosteum, connective, and epithelial tissues must be as intact as possible to facilitate nutritional transfer to flap cells. Flap perforation usually occurs when the flap is too thin or the barrier is bent. Infection and abscess formation are the main causes of failure [96, 97]. Use of antibiotics is fundamental. Rupture of a suture, gingival resection, pocket formation between membrane and flap are the other causes of failure.

5. BONE REPLACEMENT GRAFTS

The most commonly used technique for regeneration is the use of bone replacement grafts. Bone replacement grafts can promote tissue/bone regeneration via a variety of mechanisms. Some grafts actually contain cells that lay down bone matrix, ultimately resulting in new bone formation. These grafts are referred to as having osteogenic properties. Other grafts release growth factors and other mediators that signal the host to produce native bone. These grafts are considered osteoinductive. Some other graft materials might simply act as scaffolds into which the host bone grows. This property is referred to as osteoconductive [98]. There are many different sources of bone replacement grafts, each with different advantages, disadvantages, and success rates. In general, grafts can be categorized into autografts, allografts, alloplasts, and xenografts [1].

5.1. Bone Graft

Bone grafts have been used successfully to regenerate new bone in implant dentistry [99]. Autogenous graft or autograft is the tissue obtained from a site different from that of the surgery and is considered the gold standard for bone grafts [98]. Autograft can be obtained from the surrounding buccal plate, or from areas such the iliac crest or tibia. The advantage of this type of graft is the absence of disease transmission risk. However, the patient needs to be submitted to a second surgical procedure [1]. In a study that used iliac crest grafts to treat intra-bony defects, there was a gain of 4 mm bone [100]. Another study showed that the use of autografts results in true periodontal ligament regeneration with new cementum formation [41].

5.2. Allograft

Allograft refers to tissue recovered from one individual and transplanted into another individual of the same species. The grafts are

usually obtained from tissue banks that process the tissues. These materials have relatively high success rates and have an additional advantage of not requiring an additional surgical procedure to harvest autogenous bone from a donor site. Potential disadvantages include foreign body immune response, cost, and contamination of the graft during processing [1]. Studies that reported the use of this graft, demonstrated at least 50% bone fill in 67% of the treated periodontal defects, and this percentage increased when the allograft was combined with autogenous graft [41].

5.3. Alloplast Graft

Alloplasts are synthetic or inert foreign materials that are implanted into host tissue. They only have osteoconductive properties. Some examples of alloplasts are hydroxyapatite, beta-tricalcium phosphate, non-ceramic polymer or bioactive glass. This type of grafts has also been used widely in periapical surgery to enhance new bone formation [73, 101]. An example is calcium sulfate, which is an osteoconductive material but is not capable of recruiting mesenchymal stem cells from the bone marrow or endosteum and osteoprogenitor cells in the periosteum that can differentiate into committed pre-osteoblasts [102-104]. It must dissolve in the tissue fluid or integrate into bone before or during new bone formation [17]. In two studies, calcium sulfate was placed in the osteotomy sites during periradicular surgery, and it did not affect the deposition of cementum on the resected root surfaces [101, 105].

5.4. Xenograft

Xenograft is tissue obtained from a species different from that in which it will be implanted. Xenograft is totally biocompatible. Since antigenicity is a concern with this type of graft, the tissues are processed to remove the organic constituents, leaving only the inorganic matrix. This type of graft resorbs very slowly and might undergo fibrous encapsulation [106].

Xenografts yielded positive clinical results in the treatment of intra-bony defects, furcation, and endodontic-related surgical defects [107, 108]. A prospective study comparing the outcomes of endodontic microsurgery in cases of isolated endodontic lesions with those with lesions of endodontic-periodontic origin in which a combination of calcium sulfate and an absorbable membrane were applied to the periradicular defects showed successful outcome rates of 95.2% and 77.5%, respectively, at the 1 to 5-years postoperative follow-up [109]. In another case of endodontic-periodontal lesion, a bone substitute was used as a space filler to support the overlaying membrane and also potentially as a scaffold to provide osteoconductive properties for new bone formation. Preoperative radiolucency was resolved in 6 months. Also was observed a radiopaque-granular appearance, probably caused by the presence of xenograft [110].

6. Final Considerations

From this review, it can be concluded that the main objective of GTR is promote the growth of periodontal ligament and alveolar bone cells, while simultaneously blocking the proliferation of other tissues, especially epithelial and connective tissue, thus promoting osteogenesis in bone defects [20, 30, 48, 53, 111, 112].

GTR is an option for the repair, although its results are difficult to predict. This technique can increase the quality and quantity of new bone formation. Big periapical, endodontic-periodontic, and through-and-through lesions, when treated with GTR, showed excellent healing.

Barriers or membranes play the role of excluding gingival tissues from the repair area, protect the surgical wound, provide physical support to the flap, and create a space for the clot, thus favoring the growth of cells native to the defect. The results obtained until date have been independent of the type of membrane used in the GTR procedures. Hence, it cannot be affirmed whether absorbable membranes are better than nonresorbable membranes [113]. Both membranes have their own advantages and disadvantages. Membrane use is indicated in the treatment of enclosed

defects, such as bone defects or furcation defects, where the periodontal ligament cells can migrate from all the edges.

The choice of membrane depends on the clinical situation. Irrespective of the type, barriers or membranes need to possess some fundamental characteristics for their use. The ideal material should be flexible and permit the correct placement upon bone defects. However, it should be strong enough to avoid deformation. Although there is no difference in the results obtained with any of the membranes, some studies indicated that absorbable membrane should become the membrane of choice in the future, because it eliminates the need for a second surgical procedure, allows the growth and maturation of tissues, and controls infection.

The ideal duration for which the membrane should remain intact after its implantation on periodontal tissues was calculated to be 50 to 60 days. The non-absorbable barriers provide better control over this duration. The effects of using membranes and grafts together are not yet well understood. Using reparative techniques and materials, such as bone grafts, growth factors, coronal flap reposition, and platelet rich plasma (PRP) can yield better results in terms of insertion gain and bone defect fill.

Infection control is crucial to the treatment success, as well as the preparations before the surgery. The most cited complications are post-operative infection and membrane exposure.

It has been demonstrated histologically that GTR intervention results in true periodontal repair, with the formation of cementum, connective fibers, and new alveolar bone [2, 29, 34, 73, 114].

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Chapter 5

GUIDED TISSUE REGENERATION IN FURCATION DEFECTS

Gustav Guimarães[1,*], Francine Benetti[2], João E. Gomes-Filho[2], Gustavo Sivieri-Araújo[2], Rogério de C. Jacinto[2], Elói Dezan-Júnior[2] and Luciano T. A. Cintra[2]

[1]Department of Periodontics, São Lucas Faculty, Porto Velho, Rondônia, Brazil
[2]Department of Endodontics, São Paulo State University (Unesp), School of Dentistry, Araçatuba, São Paulo, Brazil

ABSTRACT

One of the indications for guided tissue regeneration (GTR) is furcation lesion. Periodontal regeneration of this type of defects is not predictable, especially in terms of complete bone fill. Furcation is the area between the roots of the teeth. Furcation defect refers to the pathological destruction of periodontal tissues of this region. It occurs because of the progression of periodontal disease, endodontic involvement, or occlusal changes. One important factor for successful

[*] Corresponding Author email: gustav@saolucas.edu.br.

regeneration at furcation sites is the amount of periodontal tissue that remains apical and lateral to the defect. Correct diagnosis will determine the ideal treatment modality. With the advent of GTR, there has been improvement in the prognosis of furcation defect treatments. This chapter reviews the current literature on the treatment of furcation defects, with particular emphasis on guided tissue regeneration.

Keywords: guided tissue regeneration, guided periodontal regeneration, furcation defects, periodontal disease

1. PERIODONTAL DISEASE AND FURCATION DEFECTS

Periodontal disease, or periodontitis, is one of the most prevalent global diseases of the oral cavity and is the major cause of tooth loss in adults [1-3]. It is a multifactorial disease characterized by the formation of a periodontal pocket resulting from initial gingival inflammation and subsequently affecting periodontal tissues, such as periodontal ligament, cementum, and alveolar bone [1-4]. The destruction of these tissues and connective tissue attachment loss are characteristics of this disease [4]. In addition to the local damage, chronic periodontal disease has an associated risk of systemic complications [1].

One of the most serious complications resulting from periodontal disease is the bone loss in the furcation region of posterior teeth [5]. This is because of the complex dental anatomy that makes it difficult to clean the area adequately during routine treatment. The residual calculi contribute to the infection at the site and progression of periodontitis [2, 6, 7]. These processes make the furcation region of multirooted teeth a unique periodontal site with important clinical and therapeutic implications [6].

Furcation defects call for complex and challenging procedures during the treatment of periodontitis and may reduce the effectiveness of periodontal therapy, due to limited access to furcal areas [8, 9]. Therefore, proper diagnosis of the furcation lesions and knowledge of furcal anatomy are essential for treatment success [9, 10].

1.1. Furcation Defects: Definition and Classification

Furcation defects have been considered synonymous with more advanced forms of periodontitis, with grimmer prognosis. The American Academy of Periodontology defined the bifurcation lesions as 'the pathologic resorption of bone in the anatomic area of a multi-rooted tooth, where the roots diverge.' They are classified according to the site of injury and therefore thorough anatomical knowledge of the area is critical for correct classification [6].

The classification proposed by American Academy of Periodontology is based on the published work of Hamp et al. 1975 [11]: Class I - horizontal loss of periodontal tissue support ≤3 mm and can be considered as mild to moderate periodontitis; Class II - horizontal loss of support >3 mm, but not encompassing the total width of the furcation, and can be considered advanced periodontitis; Class III - horizontal through-and-through destruction of the periodontal tissue in the furcation (tip of the Nabers probe visible at the contralateral furcation opening) [12-15]. When the level of the gingiva is below the furcation lesion, so that the furcation opening is clinically visible, be it Class I, II or III, it can then be classified as Class IV [13, 16, 17].

In the current literature the diagnosis of an advanced periodontitis will always occur the possibility of a compromise of the furca region. Teeth with three or more roots can prove to be more complex cases of periodontal disease, resulting in difficult treatment with poor prognosis. This is because, in such cases, other factors may be involved, in addition to the horizontal bone loss [13].

However, these various characteristics are not included in an ideal classification when it comes to furcation defects. However, they have important influence and must be taken into account during the treatment.

For successful diagnosis, two methods of examination, radiographic and clinical examination, need to be employed along with the use of periodontal probes. The radiographs can be helpful in the diagnosis of furcation defects, but they are of limited use, especially in cases of medium

or small bone defects that are often not visible radiographically. Hence, it is essential to perform a correct and reliable clinical examination.

1.2. Factors Influencing Prognosis

Among the factors that influence the treatment and prognosis of bone defects, are the external morphology of teeth, including the diameter of the furcation, the length of the root trunk, the root concavities, the cervical enamel projections and beads.

If the diameter of the furcation is smaller than the average width of a curette blade, it is not suitable for proper handling of this region [14].

The root length of the stem, defined as the area extending from the cemento-enamel junction to the area of bifurcation, influences the progression of periodontal disease. The molar roots with short trunk are more susceptible to periodontal disease. However, the prognosis for these cases is favorable owing to the low degree of bone destruction. In molars with long trunk, normally bone destruction is severe and the prognosis is unfavorable [14, 15].

The root concavities, represented by depressions, are anatomical features that complicate the treatment of this region, since they promote the accumulation of bacterial biofilm in a region difficult to sanitize. Cervical enamel projections and enamel pearls are also considered anatomical features that accumulate plaque resulting in bone defects in the furcation area [14].

Additionally, dehiscence, horizontal bone defects, the condition of the gingival and endodontic tissues, and the proximity of the roots can influence the outcome of periodontal therapy [13]. All these factors should be considered in determining the choice for regenerative therapy [13].

Clinical examination should discover whether the tooth has undergone endodontic treatment in order to identify the cause and origin of the lesion.

In the case of dental elements without endodontic treatment, the condition of the pulp tissue must be assessed by the pulp vitality tests. If the pulp demonstrates vitality, the lesion can be of periodontal origin. In

necrotic pulp, the tissue colonized by bacteria can penetrate the accessory canal in the region of the furcation causing the destruction of the fibers of the periodontal tissues and bone resorption [16, 17]. In this case, the source of injury can be endodontic and endodontic treatment should be performed. Occlusal trauma also should be considered as one of the possible causes of periodontal tissue loss. It is important to treat the initial cause before treating the furcation defect to ensure success.

2. GUIDED TISSUE REGENERATION AND PERIODONTAL DISEASE

The guided tissue regeneration (GTR) technique for regenerating lost tissue employs a physical barrier to isolate to facilitate its repopulation by cells of choice. The development of this technique has been possible because of the advances in tissue engineering, which integrates the principles of engineering, life sciences, biology, and clinical medicine to develop biological substitutes that restore, maintain, or improve tissue function [18]. GTR belongs to one of the major classes of tissue engineering techniques, the conductive technique [18]. This technique uses biomaterials to facilitate the growth or regeneration of tissue.

GTR is one of the treatment options for treating periodontal defects and regenerating lost tissue, without the need to carry out tissue grafts [19].

Until about three decades back, the only option for treating teeth with advanced periodontitis was extraction, and later rehabilitation with implants. Although the use of implants gives predictable outcomes, periodontal regenerative therapy has gained popularity because it allows the retention of original teeth affected by periodontal disease, thus yielding more satisfactory results when compared to those with rehabilitation using implants or grafts [4, 19].

If the site is contaminated, the first step in the treatment of periodontitis is cleaning the area with debridement and/or curettage to remove the deposits present on the root surface, which cause local inflammation [20]. Thus, it is possible to control the bacterial infection

responsible for periodontal changes [21]. The next step involves the regeneration of lost tissue, which is accomplished via GTR technique, to restore the original architecture of the lost periodontal tissue [22].

2.1. GTR Membranes for Use in Periodontal Tissue

There are several membranes that can be employed in the GTR technique. However, for a membrane to be considered an ideal barrier, it must be biocompatible, cell-occlusive excluding undesirable cell types from entering the sequestered space adjacent to the root surface, able to protect the space to be regenerated, able to integrate into the host tissue, clinically manageable, and available in configurations that are easy to trim and to place [1, 23-25].

The first membranes to be used for GTR, which constitute the first generation membranes, were not resorbable. An example is expanded polytetrafluoroethylene (e-PTFE) membrane, specially designed for periodontal regeneration (Gore Tex Periodontal Material) [1, 25, 26]. The purpose behind the use of this membrane, made from Teflon, is to isolate the defect of the gingival tissue, allowing the regrowth of the periodontal ligament along with the regrowth of cementum, and alveolar bone [27]. However, these membranes have certain limitations and disadvantages, such as susceptibility to exposure with subsequent possibility of infection as well as the need for second surgical intervention to remove the membrane [1, 25].

Resorbable membranes have been developed to overcome these limitations, and have improved the clinical outcomes considerably [27]. One of the main advantages of resorbable membranes is that the patient does not have to undergo a second surgery. These resorbable membranes constitute the second generation membranes developed for use in GTR, and can be divided into natural and synthetic membranes [1].

The natural membranes, made of collagen, allow a physiological induction as well as the maintenance of natural components of cells.. However, the disadvantages of these membranes include breakdown before

the expected time, thereby permitting the invasion of undesirable cells such as fibroblasts from the surrounding connective tissue into the defect site, which results in defects in the neoformed tissue [1, 25, 28]. Immune response may also be a risk factor with these membranes [1]. Synthetic membranes made from polyester are biocompatible, but are not inert, and can evoke reactions in the surrounding tissue during their disintegration [1].

Tissue engineering has developed third generation membranes, which not only act as barriers to protect the area to be regenerated, but also act as inducers of specific agents such as growth factors and adhesion factors, creating a natural environment during all phases of tissue regeneration [1, 29].

Despite the limitations of using barrier membranes in the treatment of periodontal tissue defects, their proven tissue regeneration capacity has been considered a great advance in tissue engineering for the treatment of periodontal lesions. Today, the ultimate goal of treatment is not only arresting the progression of periodontal disease, but also regenerating the lost tissue.

The literature supports the use of GTR for periodontal regeneration in two clinical situations: presence of furcation as well as intra-bony defects [27]. This chapter discusses GTR for the treatment of furcation defects.

3. GTR IN FURCATION DEFECTS

According to Hamp et al. [11], the treatment success of multirooted teeth depends on the complete elimination of the plaque retention areas from the bi/tri-furcation area and maintenance of meticulous posterior oral hygiene by the patients. Several techniques have been suggested for the treatment of furcation, including root resection, overall retail odontoplasty, scraping open field, extraction, and GTR in more recent times [30, 31].

GTR is used in furcation defects to exclude connective tissue and epithelial tissue from the defect area around the root, which is to be repopulated by the tissue existing previously, i.e., cement and bone

tissue, and to allow the reformation of periodontal ligament fibers [4, 9, 21, 23, 25, 32, 33].

Treatment success with GTR depends on adequate membrane stability, for the occurrencehealing of the flap surgery by first intention healing, and patient compliance [2, 34].

Bone grafts can be used concurrently with barrier membranes [2, 31, 35, 36]. The combination of grafting and GTR has been suggested to accelerate and improve the tissue regeneration process [37-39].

Studies have demonstrated that the use of bone graft materials in combination with a GTR barrier improves defect fill, reduces probing depth, and enhances clinical attachment gain [4, 19, 40, 41]. This is because bone grafts have osteogenic, osteoinductive, and osteoconductive properties [27], and prevent the collapse of the membrane used in GTR into the defect area. Thus, some authors recommend the combination of these techniques to improve membrane stability [39].

Bone substitutes can be organic or inorganic [2, 42]. They are classified into autografts, allografts, xenografts, or alloplasts [27]. The autograft, which is obtained from the same individual, has been considered the material that best represents the properties of osteogenesis, osteoinduction, and osteoconduction [43, 44]. However, it is difficult to obtain autograft in large quantities [43]. In contrast, allografts and xenografts are available in abundance. Xenografts are organic grafts obtained from species other than those into which they are implanted, such as bovine bone grafts for implantation in humans [4]. Allografts are obtained from a different individual of the same species. The major disadvantage in the use of allografts and xenografts is the possibility of immunorejection [45]. Alloplasts are inorganic grafts.

Using only bone grafts has had limited success in treatment of class II or III furcation defects. This is because connective tissue and/or epithelial tissue often can invade the graft, resulting in treatment failure. The absence of a barrier that retains the graft material in the defect area can also affect the outcomes.

GTR has become a treatment option in cases that were not successful when treated with bone grafts alone. It is recommended that GTR be used in combination with bone grafting.

3.1. GTR in Furcation Defects: Surgical Technique

McClain & Schallhorn 2000 [13] in their review describe the technique used for the treatment of bifurcation lesions, with or without the use of bone grafts. According to the authors, the techniques used in these cases involve the creation of a sulcular incision in envelope form, in order to ensure maximum gingival retention, while at the same time exposing the defect sufficiently, debriding the defect, planning the root surface, and ensuring thorough cleaning of the region, removing plaques and enamel projections, and making any other alterations. Odontoplasty can then be performed if necessary. Bone graft, when used, is hydrated with an anesthetic, sterile saline, or tetracycline solution, and set aside. The selected membrane is trimmed to the desired size, and set aside. The root surface is treated with citric acid, and is scraped to stimulate bleeding. The bone defect is completely filled with bone graft, the membrane is placed over the graft and the flap is positioned to cover it completely. The wound is closed with suture made from non-resorbable yarn. Antibiotic, anti-inflammatory and analgesic regime is prescribed, the patient instructed in the control of bacterial plaque and appropriate care of the area, and supervised by a professional. If using non-resorbable membranes, they must be removed 6–8 weeks after surgery, with minimal flap reflection and with maximum smoothness, and then incision sutured again.

Prathap et al. [17] did not use citric acid. After root planning, the authors applied topical tetracycline chloride, to eliminate degenerated Sharpey's fibers, bacteria, bacterial products, disintegrated cementum, and dentin from the root surfaces. For postoperative care, the authors prescribed antibiotics and analgesics.

Khanna et al. [46] have prescribed to their patients the use of 10 mL of 0.2% chlorhexidine mouth wash twice daily for six weeks. The sutures

were removed a week after the surgery. Eickholz et al. [47] also prescribe the use of 0.2% chlorhexidine gluconate and 1% chlorhexidine gluconate in cases of exposure of the membranes.

3.2. Treatment of Furcation Defects with GTR: Studies and Case Analysis

Bremm et al. [48] demonstrated a greater reduction in probing depth after treatment of class II furcation defects, when treated using GTR technique with resorbable membranes, compared to the results in the control group, which consisted of open flap debridement.

Eickholz et al. [47] compared the long-term clinical results after GTR therapy of class II furcation defects using non-resorbable and bioabsorbable barriers. Patients were randomized to GTR treatment with non-resorbable expanded polytetrafluoroethylene barrier (ePTFE) or bioabsorbable polyglactin 910 membrane. The authors evaluated the horizontal clinical attachment level (CAL-H) after 10 years, and failed to show a statistically significant difference in the stability of CAL-H gain between the two groups.

Trombelli & Farina [49] reviewed the results obtained by using bioactive agents alone or grafting in combination with GTR. These bioactive agents are growth factors used to enhance tissue regeneration by stimulating cellular processes such as chemotaxis, differentiation, and proliferation. The authors noted that the term ‘bioactive agents’ denotes a class of molecules or compounds, which may stimulate a variety of cellular events essential for periodontal regeneration. Therefore, it is likely that these bioactive agents, individually or in combination with other technologies, may be relevant to regenerate periodontal ligament, new cementum, and bone. However, currently, there is limited scientific evidence that supports the use of these materials, with or without the GTR, for the treatment of furcation defects.

Khanna et al. [46] evaluated the effectiveness of the combination of hydroxyapatite and β-tricalcium phosphate bone alloplast with

bioresorbable GTR membrane for the treatment of mandibular grade II furcation defects. β-tricalcium phosphate and hydroxyapatite belong to the class of calcium phosphate ceramics that have become available as alloplasts for the restoration of periodontal osseous defects. The authors concluded that resorbable collagen membrane had excellent handling characteristics and was biocompatible, and could be used effectively in the treatment of human grade II furcation defects. The authors also concluded that using resorbable GTR membrane in combination with bone grafting material was more effective in the treatment of furcation defects than open debridement alone.

Prathap et al. [17] performed a clinical evaluation of using porous hydroxyapatite bone graft (Periobone G) with and without collagen membrane (PerioCol) in the treatment of bilateral grade II furcation defects in first permanent molars. Hydroxyapatite bone graft is a commonly used alloplastic material, and Periobone G is a ceramic surgical implant used to treat periodontal defects. The parameters evaluated in study were plaque index, gingival index, mean vertical probing depth, mean horizontal probing depth, changes in the position of gingival margins, and clinical attachment level. They observed that using hydroxyapatite bone graft alone or in combination with GTR had significant effect on the clinical parameters at 3 and 6 months post-treatment, when compared to the baseline values. Both the methods of treatment yielded significant reduction in horizontal and vertical probing depth and gain in clinical attachment level. However, the combination technique yielded superior results compared to those obtained with bone graft alone, but the difference was not statistically significant.

Reis et al. [50], considering reports of cases where there was the collapse of absorbable membranes used during GTR technique for treating lesions of furca, developed a rigid membrane consisting of polyhydroxybutyrate (PHB), an absorbable polymer used to provide rigidity and stability of the membrane, and hydroxyapatite (HA) to increase the complexity of the membrane's surface topography. The authors conducted a study in dogs using membranes made of either 25% or 35% HA in PHB or open flap debridement alone (control group). The

authors obtained only partial regeneration of the defect because of wound contamination and concluded that rigid absorbable membranes made of HA and PHB failed to improve the regeneration of class II furcation defects in dogs.

Storrer et al. [2] proposed a technique using surgical cola N-butyl-2-cyanoacrylate (NBCA) as a barrier in the treatment of a class II furcation lesion by GTR. The authors observed a gain of horizontal bone and clinical stability. According to the authors, the NBCA has high hemostatic power, adhesive properties, and acts as an effective antiseptic barrier against infectious agents or pathogens found in the surgical environment. When it comes into contact with tissue or humid environment, it polymerizes rapidly, guaranteeing a solid adhesion to tissues. It has the ability to adapt naturally to the tissue anatomy, and is not damaged by blood or body fluids. The authors observed that the surgical cola shortens the surgical time and is effective for the stability of the graft.

A clinical-radiographic comparative study, published in 2015 by Srivastava et al. [3] evaluated the bonefill in periodontal osseous defects treated with GTR, using a combination of bioresorbable membrane (PerioCol) and graft bone (Grabio Glascera) with the usage of bone graft (Grabio Glascera) alone. The authors describe the bioactive ceramics as a group of materials that includes osteoconductive HA, fluorapatite, bioactive glass, and tricalcium phosphate, which are among the alloplastic grafts. Bioactive glass is a ceramic with a capacity to bond to the bone. In the study, the patients were divided into two groups, one treated with the combination of GTR (PerioCol) and bone graft (Grabio Glascer) containing bioactive glass and synthetic HA, and the second group treated with bone graft alone (Grabio Glascera). They observed that both groups demonstrated a significant improvement in both soft and hard tissues 6 months after treatment. Both the treatments were equally effective, allowing the authors to conclude that using GTR membrane in combination with bone graft affords no additional advantage compared to using bone graft alone.

Reddy et al. [51] conducted a review with the goal to critically appraise the effectiveness of regenerative therapy in the treatment of

furcation defects and to recommend future research in this area. The authors observed that the treatment of class II furcation defects results in periodontal regeneration, as demonstrated histologically and clinically. Although periodontal regeneration has been demonstrated histologically in treated mandibular class III defects, the clinical evidence is limited to one case report. Evidence supporting regenerative therapy in maxillary class III furcation defects in molars and premolar furcation defects is limited to clinical case reports and the outcomes were unpredictable. Regenerative therapy may be beneficial in class I furcation defects in certain clinical scenarios. However, most Class I furcation defects require no regenerative therapy. Finally, the group recommended that periodontal regeneration be established as a viable therapeutic option for the treatment of various furcation defects, and must be considered before resective therapy or extraction.

FINAL CONSIDERATIONS

The treatment of class II or III furcation defects is a great challenge for periodontists, owing to the complex anatomy of the region, which makes it difficult to decontaminate the area completely. Today, not just cleaning, but also the regeneration of lost tissue has become the objective of periodontal therapy. This is because tissue engineering offers the possibility to regenerate the periodontal tissues. This breakthrough of tissue engineering has been introduced into the clinic in the form of GTR technique for the treatment of periodontal tissues, including regeneration of gingival tissue, tissue regeneration in intra-bony defects, and tissue regeneration in furcation defects.

Although there is practically a consensus among researchers and clinicians about the effectiveness of GTR procedures in obtaining significant gains in new connective tissue attachment and the formation of cementum and bone in periodontal defects, a small number of studies report that there is no significant difference in the results obtained by treating furcation lesions with GTR technique or bone grafts alone.

However, most clinical studies report excellent results using the two techniques concomitantly.

New studies aim to identify more complex materials that exhibit strong osteogenesis, osteoinduction and osteoconduction, which can be used in combination with the GTR technique. Various types of materials are being tested. Those considered to be bioactive have been gaining popularity, because they exhibit one or more characteristics of osteogenicity, osteoconductivity, and osteoinduction.

In summary, the use of GTR technique for the treatment of periodontal lesions, including more complex lesions of furcations, is now a reality, as evidenced by the regeneration of periodontal tissues with this treatment.

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Chapter 6

GUIDED TISSUE REGENERATION IN INTRABONY DEFECTS

Cléverson O. Silva*, André B. de Souza and Vítor M. Sapata
Department of Periodontics, Ingá University Center (Uningá) and State University of Maringá (UEM), Maringá, Brazil

ABSTRACT

Treatment of destructive periodontal disease with scaling and root planning prevent disease progression, however, does not regenerate bone defects, a consequence of the disease process. New understanding of periodontal tissues, wound healing and technologies focused on regenerative procedures are improving the treatment outcomes in this clinical situation. The procedure used to regenerate bone defects around teeth is known as guided tissue regeneration (GTR), and it aims to increase periodontal attachment of affected tooth and to decrease pockets depth, with the reconstruction of periodontal attachment apparatus. This chapter aims to review the current approaches of GTR in intrabony

* Corresponding Author address: Cléverson O. Silva, Department of Dentistry, Ingá University Center (Uningá), Rod PR 317, 6114, Maringá, PR, Brazil. Tel.: +(0055) 44 3033-5009, Fax: +(0055) 44 3033-5009. Email: prof.cleversonsilva@gmail.com.

defects, demonstrating long-term effects and benefits of regeneration as well as the evidence for clinical efficacy and effectiveness.

Keywords: infrabony defect, periodontal disease, regeneration

1. INTRODUCTION

Periodontitis is a plaque-induced disease that lead to bone and attachment loss during its progression. Patients with moderate form of periodontitis usually achieve periodontal health conditions of dentition, with reduced pocket depth, after non-surgical treatment, that could last a lifetime when subjected to high oral-hygiene standards [1, 2]. However, this treatment does not induce regeneration of periodontal tissues and formation of a long junctional epithelium is expected during wound healing. However, in severe cases of periodontitis, particularly in the presence of intrabony defects and furcations, an additional surgical therapy is usually necessary [1, 2].

In the last decades, the use of regenerative treatment aimed at restored the lost periodontal support has become more common [3]. Both surgical and non-surgical periodontal procedures, for treatment of periodontal disease, result in gingival recession after healing, which can lead to poor esthetics, particularly when ressective procedures are used during treatment [1]. On the other hand, the presence of intrabony defects can result in residual pockets inaccessible for proper cleaning. Both sequelae of periodontal treatment, gingival recession and residual pocket, can be reduced or avoided using a regenerative procedure for restoring periodontal attachment and bone defects [3].

In this context, regenerative procedures are those designed to restore parts of the tooth-supporting apparatus that have been lost due to injury of periodontal tissues. Consequently, regeneration is the reconstruction of a lost part, restoring the original architecture and function of the periodontal apparatus [4], i.e.: formation of a new cementum with inserted collagen fibers and the re-growth of the alveolar bone.

The indication for the use of a regenerative procedure can be based on esthetics and functional considerations and on the improvement of long-term prognosis of the treated teeth [5]. Regenerative therapy can also be indicated in cases of localized gingival recession to obtain root coverage, leading to improved esthetics and reduced sensitivity [6, 7]. However, different techniques with superior predictability and success rates, such as subepithelial connective tissue graft, are still considered the gold-standard for root coverage [6, 7]. Another indication for regenerative therapy is furcation-involved teeth, particularly mandibular molars, with improvement of long-term teeth prognosis [8].

Many studies reported periodontal regeneration following a great variety of surgical approaches [1-3, 5]. However, in many cases, it is common to identify histologically an epithelial lining along the treated root surface instead of deposition of new cementum, even though at clinical examination they are considered successful [9].

Therefore, some criteria were established [10] to consider a regenerative procedure really successful in terms of regeneration:

I Demonstration of new cementum, periodontal ligament and bone formation, coronal to a notch in the root surface that indicates the apical extension of the periodontitis, in human histologic specimens.

II Improved clinical probing attachment and bone, demonstrated in controlled human clinical trials.

III Demonstration of new cementum, periodontal ligament, and bone formation in controlled animal histologic studies.

Hence, we herein focus on the use of regenerative periodontal therapy for the treatment of intrabony defects. However it is necessary to keep in mind that periodontal regeneration is challenging and complex. It requires formation of all periodontal tissues in a synchronic way, generating a similar form and function found in the native periodontal attachment.

1.1. Periodontal Wound Healing

The nature of the tissue that will form during healing of periodontal tissues is determined by the type of cell that repopulates root surface [11]. After periodontal therapy, root surface may be repopulated by cells derived from 4 different sources, i.e., epithelium, gingival connective tissue, bone, or periodontal ligament. Some studies were done to demonstrate the regenerative capacity of these cells [12-14].

After proper non-surgical root debridement or open flap debridement of site with periodontal destruction, epithelial lining along the treated root surface is formed [9, 15]. This process occurs because root surface is first repopulate by epithelial cells following by gingival connective tissue cells. This healing process is called periodontal repair and refers to healing where the tissues replacing the site previously treated are not the same of those before the disease, then, the architecture and/or functions of this tissue is not fully restored. On the other hand, pre-clinical [16] and clinical [17] studies have demonstrated that meaningful periodontal regeneration can be provided by the innate potential of the periodontal in appropriate conditions for optimal wound healing. However, limiting factors to the innate regenerative potential of periodontium are not clearly understood, and regenerative outcomes, without added protocols, are not a clinical reality.

The principle of periodontal regeneration is based on the process of obtaining a new connective attachment after periodontal treatment. This process involves the reestablishment of functionally oriented periodontal fibers inserted into a new cementum and alveolar bone formation [12]. Connective cells derived from the gingiva have a lack ability to form a new cementum and consequently a new fibrous attachment to a previously affected root [13]. However, the periodontal ligament has a regenerative capacity to form a new cementum and promote a fibrous attachment [14].

These findings encouraged the adoption of a treatment concept named guided tissue regeneration (GTR). The GTR rationale is based on the prevention of epithelium and connective cells migration from gingiva. For this purpouse, a mechanical barrier is placed between flap and the treated

root, giving proper time for root recolonization with cells from periodontal ligament and leading to periodontium regeneration, i.e., formation of new periodontal ligament, cementum and alveolar bone. Many technologies were developed after that, including different types of membranes, to support surgical protocols [18].

2. BARRIER MEMBRANES

As previously explained, the intrabony defects are a consequence of periodontal disease and cannot be regenerated only with scaling and root planning. These bone defects should be treated in a way to restore the attached connective tissue and alveolar bone around teeth. However, to reach these results a mechanical barrier membrane is necessary to exclude the migration of epithelial cells into the defect. Thus, since the 1980`s, biologically compatible membranes have been produced, tested and clinically used.

The first membrane produced consisted in a cellulose acetate component (Millipore), that was not clinically viable but served to establish the concept [14]. Subsequently, some basic characteristics were established for the membrane to become clinically feasible [19]: it should be made of a biocompatible material, even though only few biomaterial are completely inert, the membranes should not promote body sensitization or induce an inflammation that interferes with regeneration process and jeopardizes clinical results. The membranes have to prevent migration of epithelial and connective tissue cells and to promote gas and nutrient exchange from connective tissue into bone defect. A flexibility of barrier material is also necessary, to fit and maintain the space adjacent to root surface, but not too soft that can collapses into bone cavity.

The rationale of GTR is to create a scaffold between root surface and bone defect promoting a condition for progenitor cells from the remaining periodontal ligament to recolonize and multiply on root surface and differentiate into new cement, periodontal ligament and alveolar bone [14]. Both bioabsorbable and non-bioabsorbable membranes follow the principle

of creating a barrier for GTR, however, they present different components, clinical approaches and different configurations, each one designed for specific applications.

2.1. Clinical Evidences

Treatment efficacy should be tested through controlled clinical trials and, preferably, with randomization. However, it is well known the challenges to conduct a clinical research with long-term follow-up in large samples. Excepted from some multi-centered clinical trials, the majority of GTR studies are small clinical trials conducted in a single center. Thus, the systematic reviews with meta-analysis became important for literature to summarize the outcomes of each clinical trial and bring a consistent clinical answer about the best conduct in each clinical situation. The systematic reviews are also important to show the level of scientific evidence of different types of biomaterials and guide the necessity of new future research.

Non-bioabsorbable membranes are considered the gold-standard barriers for GTR, although the recent literature have shown no difference on long-term clinical outcomes compared with bioabsorbable membranes [20]. The main non-bioabsorbable membrane is the high-density expanded polytetrafluoroethylene (e-PTFE), which can be reinforced or not with titanium. The bioabsorbable membranes consist in a natural or synthetic barrier that is gradually degraded. The natural membranes are made of cross-linked collagen from porcine or bovine origin. The synthetic membranes are polylactic acid or copolymers of polylactic acid and polyglycolic acid, which are biocompatible and gradually degraded by body. However, during this process, some tissue reactions are expected, thus, it is important to raise the best evidences for the clinical decision-making.

There are several clinical trials evaluating the effectiveness of different types of barrier membranes. Clinical trials usually demonstrate the effectiveness of barrier membranes with some periodontal clinical

parameters and radiographic analysis such as clinical attachment level (CAL), probing pocket depth (PPD), defect filling with bone using periapical radiographs or reentry surgery, and teeth survival rates.

In a Cochrane systematic review, Needleman et al. 2014 [21] evaluated the efficacy of GTR in the treatment of periodontal intrabony defects. The authors assessed the literature of bioabsorbable and non-bioabsorbable membranes using CAL, PPD and filling of the intrabony defect with hard tissue as clinical variables. Seventeen randomized controlled clinical trials, with follow-up of at least 12 months were analysed. Fifteen studies used the GTR with barrier membrane alone and two studies associated the barrier membrane with bone substitute. The results demonstrated a mean gain of 1.22 mm in CAL, reduction of 1.22 mm in PPD, compared to open flap debridement, and a greater intrabony defect filling at reentry. The review reported some heterogeneity between the results obtained, however, it is well known that several factors can influence the outcomes of each study. At patient level, systemic conditions and local factors can influence the previsibility of the GTR and will be discussed posteriorly in this chapter.

Other systematic review [22] evaluated 26 controlled clinical trials with 265 individuals in test group (GTR) and 432 in control group (open flap debridement), with a total of 867 intrabony defects. The included studies tested e-PTFE (9 studies), collagen (3 studies) and polymeric membranes (12 studies). The results showed and additional CAL gain of more than 1 mm, compared to open flap, and no statistically significant difference between the different types of membrane.

The results of these systematic reviews clearly demonstrate the clinical benefits of GTR. However, it is important to observe the long-term clinical behavior of the new attachment level obtained with regenerative procedure. Gottlow et al. 1992 [23] evaluated 39 patients and 80 intrabony defects, with a follow-up between 1 to 5 years. From those 80 sites treated, 65 were followed for 2 years, 40 defects for 3 years, 17 defects for 4 years and only 9 defects for 5 years. The authors showed a CAL gain of >2 mm 6 months after surgery, which was maintained during the follow-ups, even with a significant sample lost. Another study [24] evaluated the long-term teeth

survival submitted to periodontal regenerative therapy. A total of 175 patients were followed-up in a period between 2 to 16 years (mean of 8 years). The CAL was at the same level or positioned coronally compared to baseline in 92% of the cases with a 15 years follow-up. Ninety-six percent of the dental elements treated survived during the study and all teeth lost occurred in smoking patients.

2.2. Non-Bioabsorbable Membranes

One of the first non-bioabsorbable membranes was the expanded politetrafluoroethylene (e-PTFE), a polymer still considered the "gold-standard" barrier for GTR. The e-PTFE membranes are inert and highly biocompatible and can be associated with titanium reinforcement. The politetrafluoroethylene is a synthetic fluorpolymeter composed by a strong bond between carbon and fluor molecules. As there is no enzyme in the body capable to degrade the carbon-hydrogen bond, the material cannot be degraded by the organism [25]. The capacity of the e-PTFE membrane to regenerate the periodontal tissues is based on principle of the membrane resistance to support the gingival flap without collapsing. Thus, the membrane`s hardness degree and geometry are important factors to obtain success. The titanium-reinforced e-PTFE promotes better stability due the additional mechanical support provided by the titanium frame.

There are several clinical investigations and systematic reviews evaluating the efficacy of the e-PTFE membranes for intrabony defects. Lindhe et al. 2010 [19] report the results from 23 studies and a total of 351 deep intrabony defects and demonstrate the improvement in periodontal clinical parameters with the mean gain of 3.7 ± 1.7 mm in CAL.

A disadvantage of non-bioabsorbable membranes is the risk of membrane exposure and, consequently, infection during healing. Needleman et al. 2014 [21] report, in a systematic review, a prevalence of membrane exposure ranging from 20% to 68%, depending on the type of material used. The membrane exposure is a complication that decreases the predictability of the GTR and requires additional visits to the office to be

solved due to the rigorous maintenance needed and eventually the use of systemic antibiotic.

Other disadvantage of non-bioabsorbable membranes is the necessity of an additional surgery to remove them, which implicates not only in an additional postoperative regimen for patients, but also increase the costs of the procedure and the risk of losing part of the hard tissue regenerated, once the flap opening lead to a bone crest resorption [26]. Thus, in order to eliminate the second procedure, bioabsorbable membranes were developed.

2.3. Bioabsorbable Membranes

Bioabsorbable membranes came proposing a GTR approach that would eliminate the second surgical stage. Currently, two main types of bioabsorbable membranes are available: synthetic aliphatic polyester and collagen matrix from different animals. Although both materials are degraded by the body, the biologic process is different with each one [27].

The synthetic aliphatic polyester includes polylactic acid or copolymers of polylactic acid and polyglycolic acid. The advantage of these material compared to animal derived membrane is the unlimited amount that can be produced under controlled conditions. These polymers are degraded by hydrolysis and eliminated from the organism as carbon dioxide and water [27].

Collagen membranes are produced mainly from the type I collagen or a combination of types I and III collagens. The source of collagen varies greatly, but it is often a combination of porcine and bovine origin. These membranes are highly biocompatible, once these collagens are found extensively in human body. Thus, some advantages, such as hemostasis, chemotaxis for fibroblasts from periodontal ligament and gingiva, weak immune reaction and direct effect on bone formation can be expected. On the other hand, these collagen membranes present unfavorable mechanical properties and are resorbed by enzymatic activity of macrophages and polymorphonuclear leucocytes which lead to a fast biodegradation [27].

The complications related with this material are mainly early degradation, epithelial downgrowth and membrane exposure.

Collagen membranes for GTR have been tested by several animal and clinical trials. In a recent systematic review, 21 clinical trials that investigated the used of collagen membranes for GTR, with and without addition of bone substitutes, were included. The results demonstrate CAL gain of 1.58 mm (95% CI, 1.27 to 1.88), without difference between studies that used or not bone substitutes [20]. However, mechanical stability is essential to avoid membrane collapses, which interferes in GTR success. Hence, in cases in which membrane mechanical stability can not be achieved during surgery due to defect configuration, a bone substitute filling is indicated to ensure space maintenance [28].

3. BONE GRAFTS

Filling intrabony defect with bone graft provides a scaffold for cell proliferation and also gives proper stability for membrane, avoiding its collapse, which would reduce the intrabony space to be regenerated. There are several experimental researches demonstrating the negative effect of membrane collapse [29, 30]. Generally, the space obtained with membrane placement influences directly on the amount of new bone formation. In the study of Cortellini et al. 1995a [31], the authors reported that the use of e-PTFE membrane reinforced with titanium lead to statistically significant CAL gain, compared to e-PTFE alone. The authors` explanation is based on the fact that membrane with titanium reinforcement could be positioned more coronally compared to e-PTFE alone, increasing the space obtained and promoting better clinical results. However, when a collagen membrane is used, the material flexibility does not allow the coronal positioning and also increase the risk of membrane collapse. In these cases, especially when there is a two or three-wall intrabony defect, a scaffold would work as a mechanical apparatus for membrane leading to a better stability and, consequently, more consistent results.

There are several types of bone substitutes from different origins for periodontal regeneration. Autogenous, allogenic, xenogeneic and synthetic bone substitutes can be found commercially and are indicated for periodontal defects. However, there are evidences concerning the clinical effectiveness of only a few biomaterials used on intrabony defects.

The studies comparing GTR associated with bone grafts versus GTR alone demonstrate similar CAL gain but greater amount of hard tissue gain at reentry surgery [21]. A bone graft with some level of evidence in the literature is the demineralized freeze-dried bone allografts (DFDBA). Three controlled clinical trials [32-34] evaluated the use of DFDBA associated with barrier membrane and compared to membrane alone (one study used collagen membrane and two used e-PTFE membrane) in deep intrabony defects. The results demonstrated that both treatments improved clinical parameters without difference between them after 6 months of follow-up. Therefore, there are evidences supporting the idea that GTR with allogeneic-sourced bone or synthetic bone do not improve the host regenerative potential and that healing process in these cases are still unknown [3].

Other bone graft that can be used for periodontal regeneration is the demineralized bovine bone matrix (Bio-Oss®). There are case-reports demonstrating CAL gain from 1.0 to 5.5 mm treating intrabony defects combining Bio-Oss® with barrier membrane. Clinical trials show that GTR associated with Bio-Oss® promotes better periodontal clinical results compared to open flap debridement, but when compared to GTR alone no difference was found between groups [35]. There are also some pre-clinical studies evaluating histologically the regenerated defect with Bio-Oss® [36, 37]. When demineralized bovine bone matrix is used under the membrane, partial regeneration occurs. The biomaterial leads to a vital bone formation around particle and also in the proximity of alveolar bone, showing osteoconductive and osteoinductive properties. However, near the root, it was found biomaterial encapsulated in a dense connective tissue with collagen fibers oriented parallel to root surface [36-38].

The use of bone substitute for GTR leads to intrabony defect filling with graft particles embedded in new formed bone and also improvement

on periodontal clinical parameters. Although it seems that only partial regeneration of periodontal tissues occurs, it is important to consider such biomaterials in certain clinical situations, especially when there is difficulty to stabilize the membrane (significant flexibility like collagen membranes) and/or it is intending to regenerate critical bone defects (two or three-wall bone defect).

4. ENAMEL MATRIX DERIVATIVE

Based on the limitations of bone graft materials, an enamel matrix derivative (EMD) was developed by Straumann® Company, commercially available as Emdogain®. The EMD is produced in gel form and is composed mainly by porcine-derived proteins called amelogenins. Such protein is responsible for periodontal attachment during odontogenesis. The idea is to induce periodontal regeneration with a simpler surgical procedure with less postoperative complications. The mechanism of EMD is not fully understood, however, evidences suggest that the exposure of cells from periodontal ligament stimulates the expressions of growth and differentiation factors, inducing periodontal regeneration [39].

There are several case reports and randomized clinical trials in the literature. The clinical performance of EMD was well elucidated in a systematic review performed by Esposito et al. 2009 [40]. In the treatment of intrabony defects with EMD, compared to open flap debridement, it was observed, in average, 1.1 mm of CAL gain and 0.9 mm of PPD reduction. The systematic review also compared EMD with barrier membrane and no difference was found between groups in terms of CAL gain, PPD reduction and tooth loss. An interesting result was the significant difference on postoperative complication rate. Only four patients from the EMD group had complications. Whereas, 59 patients treated with barrier membrane had complications, due to high rates of membrane exposures and abscesses. However, two cases from the same study showed an association between EMD and inflammatory external root resorption [40].

Another advantage of EMD use is its presentation form. The gel can be applied directly into bone defect avoiding vertical releasing incisions and extension of periosteal flap. Thus, a minimally invasive procedure can be done with shorter mesio-distal incision and minimal flap elevation, which can be associated with a papilla preservation technique. Minimally invasive procedure was introduced by Cortellini & Tonetti 2007 [41], particularly for EMD and growth factors use in periodontal regenerations. This surgical procedure improves esthetic outcomes and also decreases postoperative complications, such as pain, swelling and amount of medication taken.

The use of EMD is associated with root conditioning after debridement and before application of gel, intending to remove the smear layer. The most common root conditioner is EDTA 24% (commercially called PrefGel®). Although it has been a common clinical practice, there is no evidence that such procedure improves clinical outcomes. There are also other agents like citric acid, tetracycline HCl, phosphoric acid and fibronectin that failed to demonstrate clinical benefits at periodontal regeneration [42].

5. GROWTH FACTORS

Another treatment modality of GTR for intrabony defects is the use of growth factor, such as platelet-derived growth factor (PDGF). The use of growth factors for GTR were first tested in pre-clinical studies conducted during the early 1990s. These studies demonstrated the regeneration of bone, periodontal ligament and cementum on intrabony defects [43]. The mechanism of PDGF is based on the stimulatory effect on periodontal ligament, fibroblasts and cementoblasts, promoting angiogenesis, cell recruitment, and proliferation of bone and periodontal ligament cells adjacent to root [44].

Clinical effectiveness of PDGF is based on two separated multicenter, randomized, controlled clinical trials [45, 46]. These studies compared the use of 0.3 mg/mL of PDGF + beta tricalcium phosphate (β-TCP) to β-TCP

alone. The results of Nevins et al. 2005 [45] demonstrated only 0.2 mm more CAL gain, but 1.7 mm more bone filling comparing the two treatment types. Still, Jayakumar et al. 2011 [46] demonstrated that addition of this growth factor led to 0.9 mm on CAL gain and promoted 0.9 mm more bone filling. As radiographic improvement was more pronounced in both groups at the same time-point, these results can be interpreted as a benefit in terms of hard tissue healing, but only a subtle improvement on clinical results. It is notable that there is some evidence that PDGF can modify healing, favoring periodontal regeneration. However, some considerations related to cost, protein stability and safety are some reasons why this protein is still not used routinely.

6. SURGICAL TECHNIQUE

6.1. Modified Papilla Preservation Technique

This technique is indicated for anterior region and in cases that patients' esthetic expectations are high. Although it is a sensitive technique, it has been proved that Modified Papilla Preservation Technique (MPPT) is very effective, especially in wide interdental spaces (>2 mm at the most coronal portion of the papilla). This technique described by Cortellini et al. 1995ab [31, 47] permits a better flap closure in the interdental area and increased space for regeneration.

The MPPT consists in (Figure 1):

1. Horizontal incision at papilla base in the buccal/palatal keratinized gingiva;
2. Buccal and interproximal intrasulcular incisions;
3. Elevation of full-thickness buccal flap, maintaining the papilla over the defect;
4. Elevation of full-thickness palatal flap, including the interdental papilla, exposing the defect;

5. Defect debridement and root planning;
6. Partial-thickness flap, apical to the mucogingival junction, on the buccal flap, to release the flap and completely cover the membrane.

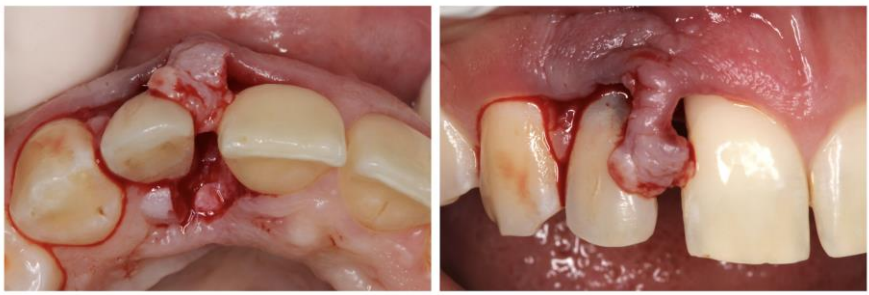

Figure 1. Occlusal and front view of incision design and flap elevation with MPPT through palatal approach.

It is noteworthy that flap should be thick to prevent necrosis, and interdental papilla must be preserved as much as possible in order to cover the membrane completely. Membrane size must surpass the defect margin between 3-5 mm.

Suture technique, to be effective, requires a supportive membrane and consists in:

1. Crossed horizontal internal mattress suture beneath mucoperiosteal flaps, between the base of palatal papilla and buccal flap, to cover the membrane and relieves all flap tension;
2. Vertical internal mattress suture between buccal aspect of interdental papilla at the most coronal portion of buccal flap to ensure primary closure

6.2. Simplified Papilla Preservation Flap

In narrower sites (<2mm at the most coronal portion of the papilla), the MPPT is difficult to apply, especially in posterior areas and when using

non-supportive membranes. Cortellini et al. 1999 [48] developed a variation of MPPT called Simplified Papilla Preservation Flap (SPPF) to overcome these inconveniences.

The SPPF consists in (Figure 2):

1. Oblique incision, with blade parallel to teeth long axis, across the defect-associated papilla, starting from gingival margin of mesiobuccal line angle of the involved tooth to reach the mid-interdental portion of papilla under contact point of adjacent tooth;
2. Buccal and interproximal intrasulcular incisions;
3. Elevation of full-thickness buccal flap, maintaining papilla over the defect;
4. Elevation of full-thickness palatal flap, including interdental papilla, exposing the defect;
5. Defect Debridement and root planning;
6. Partial-thickness flap, apical to mucogingival junction on the buccal flap, to mobilize it and completely cover the membrane.

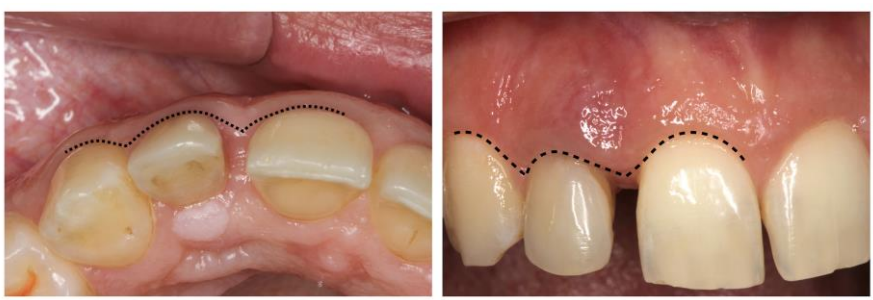

Figure 2. Schematic drawn at occlusal and front aspects of incision design of incisions for SPPF technique.

The suture technique consists in:

1. Horizontal internal mattress suture running from the base of keratinized tissue at mid-buccal aspect of the tooth not involved with the defect, to a symmetrical location at the base of palatal

flap. This suture relieves the compression at mid-portion of membrane preventing it to collapse into the defect;

2. Primary closure is obtained with interrupted sutures, or an internal vertical mattress suture, when interdental space is wide and interdental tissues are thick. No tension can be observed after complete suture process, if observed, sutures must be removed and repeated.

6.3. Minimally Invasive Surgical Technique

The minimally invasive surgical technique (MIST) is used to achieve an improvement on wound healing and decrease patient morbidity. MIST consists on use of an operating microscope (or loops at a magnification of 4x to 16x) and microsurgical instruments associated to the normal set of periodontal instruments [41, 49, 50].

The surgical technique used is the same of traditional procedure, SPPF [48] at interdental space with 2 mm or narrower or MPPT [47] at interdental sites wider than 2 mm. There are only few highlights:

1. Incisions need to be strictly intrasulcular, with minimum mesio-distal extension;
2. Elevation of a very small corono-apical full-thickness flap with the objective of exposing just 1–2 mm of the defect-associated residual bone crest;
3. Access only the defect-associated papilla;
4. Vertical releasing incisions are avoided, but when needed to eliminate tension at the extremities of the flap(s) it must be very short and not involve mucogingival junction;
5. On deep two-wall defects, larger corono-apical elevation of full-thickness flap is necessary at the site where bony wall is missing to expose 1-2mm of the defect-associated residual bone crest;

6. On deep one-wall defect, full-thickness flap is elevated to the same extent on both buccal and lingual aspects to expose 1-2mm of the defect-associated residual bone crest;
7. Periosteal incisions are never performed;
8. In very deep defects, on residual bony wall or when it involves two interdental spaces of the same tooth or two approximal teeth, flap is further extended mesially or distally involving one extra interdental space to obtain a larger flap reflection;
9. A second interdental papilla can be accessed in the latter case;
10. The use of biological agents (EMD or growth factors) is recommended.

The suture consists of a single modified internal mattress suture at the defect-associated interdental area to achieve primary closure of the papilla in the absence of any tension [49, 50] and simple passing sutures on vertical releases when needed.

6.4. Post-Operative Care

The post-operative care is vital to a successful treatment. The aim is to avoid contamination or infection of the membrane. When membrane exposure associated to infection is identified, systemic antibiotics should be prescribed (amoxicilin 1.5g/day for seven days) and a rinse with 0.12% chlorhexidine gluconate two times per day, for fifteen days is also recommended.

A weekly professional tooth cleaning with supragingival prophylaxis with a rubber cup and chlorhexidine gel should be done until the site is completely healed. Plaque control is also important. To avoid traumas on the treated site, the patients are generally advised not to perform mechanical oral hygiene and not to chew in the treated area.

When non-bioabsorbable membranes are used, they need to be removed 4–6 weeks after placement. After membrane removal, with a partial-thickness flaps, a rinse with 0.12% chlorhexidine gluconate two

times per day, for fifteen days, is recommended. Mechanical oral hygiene and chew in the treated area are avoided for 3–4 weeks. In this period, weekly professional plaque control and prophylaxis are recommended. After a completely site healing, patient can be enrolled in periodontal care program.

6.5. Complications

Membrane exposure is the most common complication, with prevalence ranging from 50 to 100% [51-61].

If the non-bioabsorbable membrane is infected, it should be removed [62]. When the exposure is limited, patient should be advised to rinse with 0.12% chlorhexidine gluconate two times per day, for fifteen days and amoxicillin 1.5g/day for seven days should be prescribed. Weekly monitoring, until complete 6 weeks for membrane removal should be done.

The exposed bioabsorbable membranes with no signs of infection can be maintained, but some care must be taken. Patient should be advised to rinse with 0.12% chlorhexidine gluconate two times per day, for fifteen days. Weekly monitoring, until complete healing, should also be done.

7. RISK FACTORS

7.1. Smoking

A smoking cessation advice should be offered before periodontal therapy. If the habit persists, patient needs to be informed that smoking habit may reduce expected outcomes, especially during healing period. A retrospective study found that cigarette smokers displayed poorly regenerative outcomes compared to non-smokers [63]. Patients who smoke more than ten cigarettes/day gained 2.1 ± 1.2 mm of CAL and non-smokers gained 5.2 ± 1.9 mm [63]. It is noteworthy that cigarette smoking displays a dose-dependent detrimental effect on CAL gains.

7.2. Periodontal Condition

Regenerative periodontal treatment is only possible after a previous basic periodontal treatment. Poor clinical outcomes have been associated in a dose-dependent manner with poor plaque control, high levels of bleeding on probing and presence of specific microbial pathogens [63-67].

Patient plays an important role in the outcome of periodontal regeneration. Patients with optimal levels of plaque control (plaque index <10%) have better CAL gains (1.89 mm greater than that observed in patients with plaque index >20%) [63, 65, 68].

7.3. Other Patient Factors

Surgery is only contraindicated when patients present severe or uncontrolled/unstable diseases. But factors like age, genetics, stress levels or systemic conditions, may be associated with poorly regenerative outcomes.

7.4. Tooth Factors

As an installed periodontal disease, unsatisfactory endodontic treatments decrease the expected outcomes or even are the cause of failure in periodontal regenerative therapy.

Another important tooth factor for periodontal regeneration is tooth mobility [69]. Hypermobility was negatively and dose-dependently associated with clinical outcomes of regeneration [49].

7.5. Morphology of the Defect

Bone gain and increase in CAL of a regenerated intrabony defect is also influenced by defect morphology. Some studies have shown a greater

CAL gain in deep defects, however, insertion gain is smaller in wider defects [64, 68, 70].

Defect width, which comprises the angle formed between defect wall and tooth long axis, can influence the final outcome. A study with 242 intrabony defects, treated with membranes, demonstrated that defects with less than 25º show better results (average 1.6mm in attachment gain) compared to defects with more than 37º [48]. However, studies demonstrated that defect with unfavorable angles can obtain higher success rate when the correct regenerative technology is associated in the surgery, like supportive membranes associated with bone replacement graft or EMD [71, 72].

The number of residual bony walls was related to the outcomes of various regenerative approaches [73, 74]. Defects with three walls tend to be more predictable compared to defects with two walls. In defect with just one wall (mesial/distal) the regenerative procedure has even lower success rates.

CONCLUSION

Regenerative periodontal procedures seems to be predictable at long-term when proper biomaterial is used. When good oral hygiene and rigorous recall program are implemented, the results of regenerative therapy can be maintained with good stability of attachment level and high long-term survival rates of teeth.

GTR with barrier membranes is the technique with more clinical evidence about its effectiveness. Non-bioabsorbable and bioabsorbable membranes are predictable in terms of CAL gain and PPD reduction compared to open flap debridement alone. However, non-bioabsorbable membranes present more postoperative complications and need a reentry surgery for membrane removal. The use of bone graft associated with membrane adds some benefits for treatment, specifically in two-wall bone defects and when a collagen membrane is used.

The use of EMD also presents benefits in terms of clinical parameters. Advantages of this material are the conservative flap opening due to its gel form, handling simplicity and less morbidity for the patient. However, bone defect anatomy is important for its previsibility, and GTR is indicated for three-wall bone defects or narrow two-wall bone defects.

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Chapter 7

GUIDED TISSUE REGENERATION IN MAXILLOFACIAL SURGERY

Francine Benetti[1,*], João E. Gomes-Filho[1], Gustavo Sivieri-Araújo[1], Rogério de C. Jacinto[1], Elói Dezan-Júnior[1] and Luciano T. A. Cintra[1]
[1]Department of Endodontics, São Paulo State University (Unesp), School of Dentistry, Araçatuba, São Paulo, Brazil

ABSTRACT

The regeneration of oral tissues depends on the body's natural capacity and the materials and techniques currently available. Bone loss in the cranio-maxillo-facial region because of trauma, anatomical or congenital causes, cancer, and bone disease requires surgical intervention. The proposed techniques include treatment with bone grafts, bone substitutes, distraction osteogenesis and guided tissue regeneration (GTR), as well as their combinations. Research in this area has been advancing. The most recent developments in tissue engineering and stem cell and gene therapy have been used in the maxillofacial surgery with good results. Tissue engineering can be divided into conductive, inductive, and cell transplantation modes. GTR is a conductive technique.

[*] Corresponding Author email: francine_benetti@hotmail.com.

This procedure, which uses biomaterials to facilitate the growth or regeneration of already existing tissue, entails using a resorbable or non-resorbable membrane that excludes undesirable types of tissue growth and permits only bone cells to populate the surgically treated site, thus guiding the whole regeneration process. Among the indications for the use of GTR technique in maxillofacial surgery are oronasal fissures requiring surgical treatment, oro-antral communication, maxillary sinus lift, increasing the height and thickness of insufficient alveolar ridge for the placement of endosseous implants and prosthetic rehabilitation, and maxillary and mandibular bone changes that require appropriate aesthetic and functional reconstruction. Depending on the size and location of the defect, various surgical techniques for bone regeneration have been described in the literature. The choice of appropriate treatment is a difficult and complex process. The technique of GTR is feasible with good prognosis, when used properly following the basic requirements for the correct application of the membranes. Over the past several years, new concepts and materials have been developed with the aim of increasing the therapeutic arsenal of professionals employing tissue regeneration therapies. This chapter will describe the use of GTR techniques in maxillofacial surgery, as well as the materials used in this procedure and different experimental models studied.

Keywords: guided tissue regeneration, guided bone regeneration, maxillofacial surgery

1. TISSUE ENGINEERING IN THE MAXILLOFACIAL SURGERIES

Tissue loss resulting from trauma, anatomical reasons or congenital abnormalities, cancer, and bone disease, is a major health concern. Besides being impaired physiologically, many of these patients are affected psychologically, especially when the damage occurs in the craniofacial region [1]. Craniofacial deformities have an enormous impact on psychosocial interactions in the lives of these patients [2]. Functional and aesthetic requirements in the management of facial surgeries have steadily increased in the last decade [3]. It is necessary to restore not only the structure and function, but also the aesthetics, to restore the self-esteem of these patients.

The regeneration in the oral maxillofacial area depends on the systemic health status of the patients, in addition to the materials and techniques currently available.

Although tissues of the oral complex exhibit regenerative capacity in response to disease or trauma, damage to these tissues can at many times be irreversible [4]. Researchers in this area have focused their efforts on developing methods based on tissue engineering and gene therapy for oro-facial reconstruction [5]. The attempts to regenerate fully functional tissue will result in multiple solutions that are beneficial for the future of craniofacial medicine [6].

Using mechanical devices, with little consideration to the effects on local cells and tissues [1], has given way to today's regenerative medicine using the techniques of tissue engineering as well as bioactive materials in combination with cells and/or biological molecules capable of inducing cell proliferation, to regenerate a functional replacement tissue at a diseased or damaged site [1].

There has been a shift from the use of materials that simply replace tissue in a non-functional manner, to that of utilizing specific materials, which will regenerate fully functional and structurally acceptable tissue.

Disfigurement and extension of a defect seen in clefts and long-lasting defects after trauma or tumor surgery, affecting speech, mastication, deglutition, and respiration, demand a restoration that is as close to normal physiological functionality as possible [3]. Synthetic materials cannot replicate the physiological properties of the original tissue [4].

Thus, the simple replacement of fully functioning sets with metal prostheses in the 60s, when the materials were thought to be 'inert', has made room to materials that are physically, chemically, and biologically capable of effectively replacing the damaged or diseased tissue with repaired or regenerated tissue [1].

The discovery of stem cells and developments in cellular and molecular biology have led to new therapeutic strategies for the regeneration of tissues that were injured by disease [3].

Tissue engineering is a multidisciplinary field that involves the principles of engineering and life sciences, biology and clinical medicine, for the development of biological substitutes that restore or improve tissue function [3, 7]. It is based on the fundamental principles of identifying appropriate cells, developing scaffolds and identifying the morphogenic signals required to stimulate cells to regenerate a tissue [3]. There are 3 major classes of tissue engineering techniques: conductive, inductive, and cell transplantation [4, 7].

Cell substitutes allow the replacement of only those cells that supply the needed function [7] and involve direct introduction of tissue that has been manipulated earlier *in vitro*. An example is cell transplantation, which involves taking existing tissue from the patient, isolating and multiplying the desired cells, and inserting them back into the patient at the defect site, to bring about tissue regeneration [4]. Limitations of this method include failure of the infused cells to maintain their function at the transplantation site [7].

The approach of using inductive substances depends on the purification and large-scale production of appropriate signaling molecules, such as bone morphogenetic proteins and platelet-derived growth factor [4], and the development of methods to deliver these molecules to the target sites [7], to stimulate surviving cells and promote regeneration [4].

The conductive technique, such as guided tissue regeneration (GTR), uses biomaterials to facilitate the growth of existing tissue or regeneration of new tissue [4]. In closed systems, a cell occlusive membrane that is permeable to nutrients and wastes but keeps out large entities such as antibodies or immune cells that destroy the transplant, is used. In open systems, cells attached to matrices are implanted and get incorporated into the body [7].

The choice of the method for regenerating lost tissue depends on factors such as the size of the defect, supply of cells from adjacent areas, cell migration rate, and presence of vasculature in the surrounding areas [6].

When the clinical need is the regeneration of a small amount of tissue, conductive and inductive techniques are utilized to recruit cells from host tissue into a scaffold, whereas regeneration in large defects often requires the direct transplantation of cells. If cells are not present in the surrounding tissue in sufficient quantities to populate the defect, depending solely on cell migration would require an unacceptably long healing time [6].

As early as in 1993, Langer & Vacanti [7] discussed the future of tissue engineering research. Researchers need to continuously explore various aspects of cell biology, regarding the causes of cellular differentiation and growth, and the relationship of the extracellular matrix with cell function. The study of molecular genetics will lead to the design of cells and materials that are not rejected by the immune system.

The ultimate goal of tissue engineering is to engineer virtually every type of tissue, including nervous tissue, cornea, skin, liver, pancreas, tubular structures, cartilage, bone, muscle, and vascular tissue [7].

2. INTRODUCTION TO GTR IN MAXILLOFACIAL SURGERY

GTR, based on the principle of in situ tissue regeneration, also termed "endogenous regeneration," stimulates the intrinsic potential of a tissue to heal or regenerate [8]. GTR, a conductive approach uses a natural or synthetic matrix that acts as a scaffold or barrier on which cells can attach, proliferate, migrate, and differentiate [6]. The ultimate aim of the regenerative therapy is the restoration of lost tissue form and function.

In 1980, Nyman et al. [9] developed the concept of GTR based on the principle that specific cells would contribute to the formation of specific tissues. Melcher [10] described in 1976, the concept of repopulation of defects by specific cell types to enhance healing. Exclusion from the defect site of tissues that grow faster than bone tissue, such as epithelium and connective tissue, facilitates the growth of desired tissues such as bone, into the defect site [11].

In GTR, a resorbable or nonresorbable barrier membrane is used to prevent the invasion of the defect site by undesirable cells [4]. Non-resorbable membranes require a re-entry procedure to remove them, and are prone to exposure and infection. In contrast, resorbable membranes do not require re-entry. However, these are frequently used in combination with grafts that provide support to the membrane [4].

Thus, GTR, a conductive technique, uses membranes alone or a combination of membranes and bone grafts, to guide the formation of new tissue. It can also employ inductive methods and bioactive scaffolds, which stimulate cell migration and proliferation [6, 12]. Various growth factors can also be used in this procedure.

The scaffold supports the proliferation of cells in the remaining tissue while it breaks down over time. The membrane allows the proliferation of desired cells in the defect site, and excludes undesirable cells. The growth factors, if used, their rate of release at the defect site as well as the quantity should be controlled. It is also necessary to control the rate of membrane degradation to ensure space maintenance long enough for new tissue formation.

Bone graft scaffolds represent the extracellular matrix (ECM), and the membrane isolates the necessary cells to proliferate and regenerate lost tissue.

Understanding of the development of tissues and organs during embryogenesis and the mechanism by which they remodel and maintain their functionalities are the prime sources of information for designing synthetic ECM used in tissue engineering and regeneration [13].

The synthetic ECMs provide structural support to tissues and play role in controlling cell adhesion, migration, growth, and differentiation via intracellular signaling pathways [13].

In the conductive technique, synthetic ECMs maintain space to allow host cell infiltration into the tissue defect site [13]. The synthetic ECMs are often used as depots of growth factors and control their release at the defect site. Similar to native ECM molecules, adhesive signals can be incorporated into the matrix to control cell migration from the surrounding tissue into the defect site [13].

2.1. Types of Membranes Used in GTR

Many factors contribute to a successful GTR outcome, including adequate barrier properties, biocompatibility, space maintenance, cell-occlusiveness, tissue integration, and clinical manageability [14].

Biomembranes are used as barriers to prevent the invasion of the defect site by undesirable cells and allow the slow migration of cells to restock the area [15]. Numerous barrier membranes have been developed, and can be grouped into resorbable or non-resorbable varieties.

Resorbable membranes are natural or synthetic polymers. Most popular ones are collagen and aliphatic polyester membranes [13, 14].

Among the non-resorbable membranes, expanded polytetra-fluoroethylene (e-PTFE) and titanium mesh are the most widely used barriers [14].

Membrane e-PTFE is a practically inert substance that exhibits a low coefficient of friction, impermeability, and low adherence [15]. These membranes provide an effective barrier function in terms of biocompatibility and can maintain the space beneath the membrane for a sufficient period, beyond which they have a reduced risk of long-term complications [14]. All these advantages overcome the major disadvantage of the necessity for removal using a second surgical procedure [14].

Another nonresorbable material applicable for dental bone repair is titanium mesh, which was previously studied by Boyne et al. in 1969 [16], for the reconstruction of large discontinuous osseous defects [14, 16].

Titanium has been used extensively because of its high strength and rigidity, low density and weight, ability to withstand high temperatures, and resistance to corrosion [14]. According with Degidi et al. [17], titanium mesh is helpful for maintaining space in large mandibular and maxillary defects [17, 18].

However, to eliminate the need for a second surgery to remove the membrane, resorbable membranes, which should be fully absorbed, must be used without causing major damage during the healing process.

In principle, stiff resorbable membranes promote a similar degree of bone regeneration and bone formation as nonresorbable membranes [14].

Collagen is the main structural macromolecule of the human body and can be used for membrane fabrication via multiple methods. However, native collagen is degraded within a few days, and untreated collagen membranes lack stability to maintain space if a bony support is missing. To overcome these problems, various crosslinking techniques have been developed, leading to the development of stiffer collagen membranes and slowing enzymatic degradation [19].

Polyglycolide or polylactide (aliphatic polyesters) can be prepared in large quantities and allows for the creation of a wide spectrum of membranes with different physical, chemical, and mechanical properties [14, 20].

3. GTR IN OSSEOUS OR GUIDED BONE REGENERATION

The shape and contour of the head region depends on the mechanical integrity of bone tissue and functions to protect the soft tissues of the cranial cavity [6, 21]. Approximately 800,000 bone grafting procedures are completed each year in the United States of America [22], and restoring or enhancing the repair of bone is a crucial problem in orthopedics and dentistry [13].

Advanced atrophy or jaw defects, cleft palates, bony nasal pyramid defects following removal of a fistulous tract or cyst, extreme deficiency of the chin, scoliosis of the mandibular arch, mandibular asymmetry, and defects following removal of sinus and mandibular tumors all require the generation of novel bone tissue [23]. Car accidents, sporting activities, and gunshot wounds can also result in blowout fractures of the orbital floor, orbital rim fractures, craniocerebral trauma, malunited fractures, major fractures of the maxilla or mandible, osteoradionecrosis, and dento-alveolar trauma [6, 23].

Various attempts have been made to reconstruct craniofacial tissues, including application of bone or bone substitutes as autogenous grafts, allogeneic grafts, xenografts [4], demineralized bone matrices, synthetic bone pastes, semisynthetic scaffolds, and various alloplastic materials.

Although each method can achieve the objective of tissue restoration, these methods all have limitations, such as donor site morbidity, graft resorption, contour irregularities, insufficient autogenous resources, disease transmission, major histoincompatibility, graft-versus-host disease, immunosuppression, structural failure, stress shielding, and infection by foreign material [2].

Among the graft materials used for bone regeneration, autogenous bone is considered an ideal material because it shows increased capacity for bone formation, osteoconduction, and osteoinduction; it does not induce immunological rejection; and heals rapidly [24, 25]. Additionally, autogenous bone grafts are limited by the inability to harvest large amounts of tissue [24], significant bone resorption, harvesting difficulties, donor site pain, and poor contouring [6, 23]. Several types of allografts and xenografts are available; however, disease transmission and immunorejection remain substantial obstacles to their implementation [6]. Synthetic bone replacements are also utilized; however, these materials generally fail over time and may show problems with biocompatibility [6, 13].

Thus, engineering tissue with the ideal features may allow formation new bone tissue through osteoconduction, osteoinduction, and cell transplantation strategies [6, 13].

In extensive reconstruction, transfer techniques for vascularized osseous bone are often used and include osseo- and cutaneous-free flaps, which are primarily applied in oncologic surgery of the head and neck region [3]. Additionally, bone block transplantation is used in cases of advanced atrophy or jaw defects before application of surgical preprosthetics to enable patients to be fitted with implants [3]. This is a necessary surgical step in the extra-oral region because of need for large-volume grafts, such as grafts in the fibula, scapula, and iliac crest, causing donor-site morbidity [26, 27]. To achieve adequate bone regeneration, adequate scaffolds and appropriate cells of the annex are needed, showing adequate proliferation and differentiation [3].

Osteoconductive approaches using engineering tissue may allow infiltration of osteoprogenitors from the bone marrow into local defects

while providing temporary mechanical support; this is called guided bone regeneration (GBR) [13].

Through a combination of transplanted biomaterials, stem cells, an appropriate mixture of regulatory factors that stimulate cellular growth and proliferation, and extracellular matrix components to allow the growth and specialization of the cells, important clinical applications in alveolar bone surgery and facial skull surgery may be achieved [3, 28].

Anatomical sealing has been employed in periodontics to allow regeneration of materials providing tooth support, i.e., the GTR. By covering the tooth roots of monkeys with membranes, Gottlow et al. [29] observed that the membrane and root were involved in bone regeneration and concluded that the principles of GTR can be used in GBR.

In addition to the membranes, scaffolds can also be used for GBR. For example, scaffolds of synthetic extracellular matrix can promote osteoconduction according to the distribution of pore diameters. Thus, the surface in contact with the bone tissue requires the largest pores, allowing cell conduction to occur more easily. In contrast, the surface in contact with fibroblasts requires smaller pores to inhibit cell proliferation. Therefore, the material blocks undesired cells types, while allowing cell types that can form bone to migrate into the site [13].

Utilization of growth factors may enhance the utilization of grafts. Urist demonstrated the capabilities of demineralized bone matrixes to form bone using rat muscle, proposing that growth factors can induce bone formation independently of the bone tissue condition [18].

To date, researchers have identified many growth factors, including bone morphogenetic protein (BMP) and growth factor beta (TGF-β). BMP is an osteoinductive growth factor that reduces the need for autogenous bone grafts [18] and has an essential role in the regulation of bone formation, maintenance, and repair [4]. Among these roles, recombinant human BMP-2 has been approved for use in sinus augmentation [4] and for localized alveolar ridge augmentation for defects associated with extraction sockets [18].

Platelet-rich plasma (PRP) is a platelet concentrate that contains a number of growth factors, including TGF-β. PRP can be prepared chair

side with minimal complications because PRP is an autologous material. Utilization of PRP has been shown to have varying effects, ranging from no effect to significant enhancement of clinical attachment. However, most studies have consistently shown that PRP results in more rapid healing, less postoperative pain, and less membrane exposure [4].

Growth factors and PRP will be described in greater detail in later chapters.

3.1. GBR in Maxillary Sinus Floor Elevation Surgery

Therapy through implantology has provided an alternative to edentulous patients. Alveolar bone loss associated with periodontitis and alveolar atrophy after tooth extraction in adults, which can result in pneumatization of the maxillary sinus, causes severe vertical and horizontal bone loss in the posterior maxilla [30]. Thus, the placement of dental implants in partially or totally edentulous posterior maxilla remains a challenging procedure owing to the small amount and poor quality of bone.

Although the use of short or tilted implants has been demonstrated to be a suitable therapeutic option, showing long-term biomechanical stability of prostheses, sinus grafting has emerged as a good option for facilitate implant placement. Maxillary sinus floor augmentation is the most frequently used method to increase the alveolar bone height of the posterior part of the maxilla. Several types of bone grafts have been studied and used for maxillary sinus floor augmentation.

Different grafting techniques have also been proposed; the lateral approach is the most commonly used technique [31]. Introduced by Tatum [32] and later modified [33,34], this approach accesses the maxillary sinus through the creation of a bone window in the lateral sinus with a round bur and maintains the sinus membrane intact; this structure is thenelevated and mobilized with placement of the grafting materials [31].

Alternative techniques have also emerged, including elevation of the sinus membrane with a crestal approach using osteotomes, and the position

is maintained by implants [32, 35, 36]. Lundgren et al. [37] showed that mere lifting of the sinus membrane without graft placement created a void space, allowing the formation of blood clots, resulting in late formation of new bone in accordance with the principles of GTR [31, 38, 39].

Based on these findings, Cricchio et al. [31] performed a study in 2011 in which they elevated the maxillary sinus membrane and realized the simultaneous insertion of titanium implants without the use of any grafting material, as described by Lundgren et al. [37]. Thus, a bone window was created in the lateral wall of the sinus, and the bone flap was removed into saline solution. The sinus membrane was elevated in order to create a secluded compartment for the implants. After elevation of the membrane, the implant was positioned, and a twist drill was used for the final preparation of residual bone. The removed bone window was then repositioned and, if necessary, secured with the aid of cyanoacrylate tissue glue. Finally, the mucoperiosteal flap was reattached.

Of the 239 implants inserted, 50 were inserted entirely in residual bone, and the remaining 189 protruded into the maxillary sinus. Of these 189 implants, 179 protruded at least 4 mm in the created sinus compartment (mean: 8 mm, range: 4–13 mm). After 96 sinus membrane elevations, the authors suggested that mere elevation of the maxillary sinus membrane at the time of implant insertion may be a successful approach for bone reformation and implant survival, consistent with other studies [37, 40, 41-43].

Thus, Cricchio et al. [31] utilized the principles of GTR and GBR without utilization of the membrane. However, the bone flap, which was removed from the bone window, was used in a protective role.

When teeth are adjacent to the edentulous area, bone augmentation is required, and the procedure becomes more difficult. The antral membrane balloon elevation (AMBE) technique is useful in this area. This technique is realized with a limited incision, minimal mucoperiosteal flap reflection, and a small window, in which the membrane is elevated to the medial wall of the sinus cavity, avoiding sharp dissection around the roots of the adjacent teeth [44]. Thus, complications, such as morbidity, blood loss, long operative time, and postoperative pain, are reduced [44].

Solta and Smiler [45] described the AMBE technique. A crestal incision is made, extending the length of the edentulous area. Osteotomy of the buccal bone is performed, and the sinus membrane must be preserved. The resulting bony fenestration is gently pressed inward, carrying the underlying membrane along with it. The dissection should progress all the way to the medial wall of the sinus. A balloon of latex material is placed against the sinus floor midway between the lateral and medial walls and is gently inflated with 2–4 mL sterile saline. As it expands, the membrane is elevated. Thus, the fragile epithelium will be subjected to minimal trauma. The balloon is then deflated and removed. A resorbable collagen membrane is soaked with PRP and placed under the elevated sinus membrane. A grafting material can then be utilized and mixed with PRP. A second guided bone regenerative membrane is trimmed, moistened with PRP or aqueous antibiotic, and placed over the lateral wall window. The mucoperiosteal flap is repositioned and sutured [45].

Among the advantages already cited, such as elevation of the sinus membrane with minimal risk of tearing and reducing postoperative pain, bleeding, and infection, this technique can be completed within 30 min, as reported by Soltan and Smiler [45]. Thus, this technique, which uses elevation of the sinus membrane, placement of the grafting material with PRP, and membranes to guide regeneration, has become a highly successful and reliable procedure [45].

As described above, application of growth and differentiation factors can be used to enhance the wound healing process. Platelets produce and release PDGF, which enhances osteogenic differentiation and bone repair in fracture models and critical-sized calvaria defects [46-48]. Additionally, PDGF has a significant role in the wound healing process with the capacity to stimulate chemotaxis, cellular activation, and proliferation in fibroblasts and osteoblast-like cells. Therefore, considerable interest has emerged because of the potential benefits of using PRP, a highly concentrated form of platelets.

In a case-series report, Kassolis et al. [48] described the use of PRP in combination with freeze-dried bone allografts (FDBAs) for alveolar ridge and maxillary sinus GBR procedures. The authors created full-partial

thickness flaps that were elevated at both the sinus and the ridge augmentation sites, and the sinus cavity was exposed for graft insertion. Maxillary sinus grafting was accomplished using the Caldwell-Luc approach. The composite graft was a mixture of FDBA and PRP, saturated with an autologous thrombin-rich extract. After the placement of the graft, coagulated PRP gel was used to cover the graft. Ridge augmentation sites required added flap mobilization and repositioning of the facial mucoperiosteum to permit passive primary closure over the FDBA/PRP.

In all three cases, the authors observed new bone formation, where FDBA particles were amalgamated with the newly forming bone. Consistent with previous studies [49], these data suggested that the use of PRP may allow for earlier implant placement and/or loading by stimulating and enhancing the wound healing process, including osseous regeneration. Bovine thrombin has been used to activate coagulation and precipitate gel formation.

One of the problems with the utilization of allografts is that osseous wound repair occurs more slowly than that with autogenous bone grafts. The degree of revascularization of bone allografts also appears to be slower than that with autogenous grafts. Utilization of these materials that may improve osseous wound repair following regenerative procedures using allografts, similar to the use of PRP. Thus, an alternative to autogenous bone grafting, which would require a second surgery for graft removal, would be allografts and PRP.

The use of bone inductive growth factors may enhance the development of mature lamellar bone, which could support dental implants sooner and more predictably. Recombinant human PDGF (rhPDGF-BB) has been shown to improve regeneration, as previously described in this chapter.

Urban et al. [50] demonstrated the successful use of rhPDGF-BB in conjunction with autogenous bone, anorganic bone mineral, and barrier membranes to reconstruct severe alveolar bone defects in the posterior maxilla. A patient with a history of dentoalveolar infection, several fistulous tracts, and advanced mobility showed advanced periodontal bone loss with associated periapical lesions upon radiographic examination. In

this case, the teeth were extracted, and a severe vertical ridge defect was noted 2 months later.

After adequate incision, a rectangular antrostomy with rounded corners was created at the lateral wall of the maxillary sinus. The sinus window was infractured, and the sinus membrane was elevated with care to prevent perforations. The recipient bony bed was prepared with multiple decortical holes to expose the medullary space. Autogenous bone was harvested from the right ascending ramus, mixed with anorganic bone mineral, and hydrolyzed in rhPDGF-BB. The e-PTFE membrane was fixed on the palatal side, and the bone graft then was placed within the subantral space and appositionally on the vertical alveolar defect. The membrane was folded over onto the buccal alveolus and fixed with additional titanium pins.

The mesial border of the e-PTFE membrane was placed 4 mm from the distal surface of tooth and was unfilled for about 2 mm supracrestally. This area was filled with additional bone graft material, connecting the denuded root surface to the open end of the membrane. A resorbable collagen membrane was applied to protect and contain this area. An additional collagen membrane was applied to cover the sinus window. Suturing was performed for tension-free primary closure.

Bone growth was evident over the membrane in the area where the resorbable collagen membrane was utilized. After removal of the titanium pins and the e-PTFE membrane, the authors observed complete vertical bone regeneration [50].

In another study [51], a 100% success rate was observed when placement surface implants were realized after vertical GBR. Thus, this treatment modality may allow bone harvesting to be avoided altogether.

3.2. GBR in Oro-Antral Communication (OAC)

The large volume of the maxillary sinus, which can be in close proximity with some tooth apexes under certain circumstances, facilitates pathological communication between the oral cavity and the maxillary

sinus, i.e., OAC, and this clinical complication is often encountered by oral surgeons [52]. The condition mostly follows dental extraction or endodontic treatment, and the intra-operative diagnosis of OAC is usually based on the Valsalva maneuver [53] or penetration of a blunt-edged Bowman probe to assess perforations of the maxillary sinus floor [54-56]. This can be contaminated by bacteria, resulting in infection, impaired healing, and chronic sinusitis. OAC may progress to the formation of oro-antral fistula (OAF), when the epithelium is involved [57]. Small fistulae tend to heal spontaneously after formation of blood clots and secondary healing [56], whereas larger fistulae rarely heal. Surgery is indicated if a fistula does not heal within 3 weeks [57], in order to avoid infection and fistula formation [56].

Many techniques have been proposed to resolve this condition. However, the selected technique should achieve both hard and soft tissue closure, repair OAC, and facilitate prosthetic rehabilitation through the placement of an endosseous implant [56]. Ogunsalu described this technique for the first time in 2005 [52].

The techniques described above have some limitations, including reduction in the depth of the vestibular sulcus, achievement of soft tissue closure only, requirement of bone grafting, and induction of severe pain and scarring in the palatal flaps.

Notably, the Bio-Oss-Bio-Gide Sandwich technique described by Ogunsalu [52] excludes all of the above limitations and has additional advantages of concurrent bone tissue regeneration, which will enable the later placement of an endosseous implant. Bio-Gide is a pure collagen membrane extracted from pigs and has a porous surface facing the bone, which allows the in-growth of bone-forming cells and the formation of a dense surface facing the soft tissue, which will prevent the in-growth of fibrous tissue into the bony defects. The membrane is made of type I and type III collagen and will resorb within 24 weeks. Additionally, Bio-Oss is a safe bone graft material processed from bovine sources with high similarity to human bone.

The technique utilizes sandwiches of Bio-Oss granules in Bio-Gide, which is sutured together at three sides. After insertion of the Bio-Oss, the

fourth side is adequately closed using resorbable sutures, creating a closed sandwich. The sandwich has a smooth side oriented upwards and a rough side positioned to face the alveolar bone. The mucoperiosteal flap is raised, and the prepared sandwich is tucked into the OAC in such a way that it forms a convexity towards the sinus and a concavity towards the alveolar bone. Bio-Oss is added to fill the concavity. The height of the alveolar ridge is substantially reduced at the site of the opening. Edges of the soft tissue to be approximated are prepared such that raw surfaces will be in contact with each other, and suturing is carried out without tension [52, 56].

In a case presented by Ogunsalu [52], after 8 months, a new maxillary sinus and subantral bone of good quality and height were created, permitting the placement of an endosseous implant. This sandwich technique at the closure of OAC is new and promising, without the need for donor site surgery, providing advantages in terms of time and cost. By using the membrane to isolate the bone tissue, this technique allows the formation of new bone tissue and achieves soft tissue closure [52].

After reporting of the Ogunsalu technique [52], Sandhya et al. [56] performed a study with 10 patients and a 6-month follow-up to investigate both soft and hard tissue closure of OACs with the use of resorbable GTR membranes and bone substitutes and application of human freeze-dried mineralized bone as grafting material. The authors did not observe significant immediate complications. Pain was present only in three patients, and swelling was present in four patients; these side effects gradually subsided by the end of day 7. Epistaxis was observed in one patient on day 1 postoperation. Delayed complications were not observed, and there was no evidence of infection, wound gaping, fistula formation, loss of graft, or sinusitis. An average bone formation of 11.84 mm was observed after 6 months, and the average width was 6.9 mm. In relation to the quality of bone formation, by the end of the 6-month follow-up, seven patients showed trabaculae indistinguishable from the adjacent bone [56].

Ogunsalu et al. first used this technique in 2000 [58], and in their classic papers, the authors suggested other possible applications of this technique to include reconstruction of the orbital floor, closure of oro-

antral fistula, reconstruction of bony cleft defects, and mastoid ablation [56, 58].

3.3. GBR in Treatment for Alveolar Cleft

One of the most common congenital anomalies is orofacial clefts, with a prevalence of 1:1000 and 0.4:1000 for complete unilateral and bilateral cleft lip and palate, respectively [59]. Patients with this deformity require multiple surgical procedures over long periods by multiple specialists in various fields [60]. The patients must undergo surgery to balance functional and esthetic outcomes against the potential increased restriction of normal maxillary growth and development [61].

The final aim is to rehabilitate these patients with missing teeth using dental endosseous implants, which requires adequate alveolar bone volume at the appropriate anatomic position [60].

Currently, the standard treatment for alveolar cleft repair is grafting with autogenous bone [60]. Although other sources of autogenous bone have been attempted, autogenous bone grafting from the ilium is considered the gold standard [59] owing to its abundance and ease of access [61].

However, this site can produce a considerable degree of postoperative morbidity, including persistent pain, prolonged recovery time, hemorrhage, scarring, and lesions of the lateral femoral cutaneous nerve [59]. In addition, iliac crest bone grafting has been shown to result in total mean volume losses of 43.1% [62] and 49.5% [63] at approximately 1 year after the secondary alveolar cleft repair. This resorption may prevent successful placement of endosseous implants without further grafting [60].

Thus, Le and Woo [60] reported the successful utilization of mineralized human allografts to treat two adult patients with severe alveolar cleft defects using a GBR technique. The treatment consisted of completely exposing the cleft defect. In case 1, in which the patients had a congenital unilateral cleft palate and large fistula on the buccal vestibule of the area around the alveolar cleft site extending into her right nostril, the

nasal layer was closed primarily by suturing, and a resorbable collagen membrane was placed to reinforce the recreated nasal lining; mineralized human allograft material mixed with the patient's blood was then placed into the cleft defect.

In case 2, a patient with a congenital cleft lip underwent multiple previous surgeries, including alveolar cleft repair with iliac crest bone grafting. Panoramic radiographs and computed tomography scanning showed a large alveolar defect at the site of the missing incisor, making it unsuitable for implant placement. Mineralized human allograft material mixed with a BMP-soaked collagen sponge and the patient's blood was then placed into the cleft defect. A resorbable collagen membrane was placed over the graft material, and in both cases, the oral mucosal layer was closed in a tension-free manner. Re-entry at 5 months was realized and showed dense bone where the graft was placed, with later placement of the endosteal implant. After 3 months, good osseointegration was noted, and the alveolar bone height and width remained stable.

Thus, the repairs were accomplished with a GBR technique without the use of autogenous bone, suggesting potential applications in the treatment of patients with alveolar clefts defects, without the need for iliac crest bone grafting and associated morbidities.

Bone grafting of the residual alveolar cleft is now a well-established technique. However, Kawata et al. [64] reported the closure of an alveolar bony defect without bone grafting, using a nonabsorbable membrane.

A patient with unilateral and nonsyndromic cleft lip or palate on the left side had late mixed dentition with good soft tissue closure. The upper left permanent canine was present, adjacent to a narrow bony cleft at the height of the apex of the neighboring central incisor. The oronasal fistula was excised, and the alveolar cleft was completely exposed. Subsequently, the nasal mucosa was carefully dissected from the oral mucosa, sutured, and pushed upwards into the nasal cavity. An e-PTFE nonabsorbable membrane barrier was placed to cover the bony defect. The membrane was pushed under the periosteum and secured passively at the base of the flaps. The flaps were then repositioned and sutured to cover totally the membrane. Four months after surgery, radiographs showed a bony bridge

and closure of the alveolar cleft. This was the first study to evaluate the use of GBR with a nonabsorbable membrane to close the alveolar cleft when the bony cleft was large and lacked bone support, indicating the requirement for the supporting membrane [64].

3.4. GBR in Reconstruction of Defects after Tumor Resection

GBR can also be used to reconstruct bone defects after tumor resection. Reconstruction of these defects represents a challenge in head and neck reconstructive surgery.

Vitkus and Meltzer [65] described a patient with adenomatoid odontogenic tumor (AOT) combined with calcifying epithelial odontogenic tumor (CEOT); this presentation was referred to as combined epithelial odontogenic tumor. Approximately 1 year after surgery for removal of an impacted canine, the patient presented with an erupted maxillary left canine with an asymptomatic firm, red swelling circumscribing the buccal and palatal aspects of the tooth and extending from the keratinized tissue to the mucosa. Intra-oral radiographs revealed a well-circumscribed radiolucency superimposed on the entire root. Buccal and palatal flaps were raised, exposing a cystic-type lesion that was easily removed and leaving a large cavity exposing the root just short of the apex. A thin layer of bone covering the root surface was found, with large vertical defects interproximally. To prevent the ultimate destruction of the tooth, a graft of demineralized freeze-dried bone was placed in the cavity and covered with a nonresorbable barrier membrane made of e-PTFE. Eight weeks later, the membrane was removed, and an intra-oral radiograph showed trabecular bone.

The literature suggests that only the lesion should be surgically excised, with little mention of whether this will result in complete regeneration of the bone and how long this process will take. However, it is unclear whether complete regeneration will occur given the large bone loss, and it is expected that even partial regeneration will take considerable

time. Despite this, the choice of GTR with freeze-dried bone grafting was made in an attempt to increase the predictability of the result.

Thus, the results showed that GTR combined with bone grafting could be used to aid in the rapid filling of large defects surrounding the teeth, created after surgical removal of the odontogenic tumors.

The quantity of bone available for harvesting may be insufficient for larger defects. rhBMP-2 is an osteoinductive growth factor that reduces the need for autogenous bone grafts. To date, studies of the use of rhBMP-2 for maxillofacial reconstruction have been promising [18].

Cicciu et al. [18] investigated the clinical and radiographic long-term results of a hemimandibular reconstruction using rhBMP-2, an absorbable collagen sponge, and titanium mesh. The patient in this case presented a large tissue mass and extension in the left mandibular region, with ominous growth; this lesion was diagnosed as a dentinogenic ghost cell tumor. The decision was made to manage the local tumor with en bloc resection and immediate reconstruction using an inferior titanium plate in order to maintain the mandibular soft tissue space. This process was carried out using an incision that extended into the bone and subperiosteal dissection to expose the entire defect. Subsequently, a mesh was placed to correct the defect. The underlying ridge was pierced to stimulate bleeding, allowing faster integration of the graft and supplying stem cells to the area. The rhBMP-2 was added to the absorbable collagen sponge, and a portion of the sponge was then cut into small pieces, mixed with the bone allograft, and placed into the titanium mesh. The titanium plate was subjected to maxillomandibular fixation and locked with the inferior plate for mandibular reconstruction.

After 3 or 4 months, the patient exhibited radiographic evidence of bone formation, and mandibular continuity was regained, as demonstrated both clinically and radiographically. After 9 months, the titanium mesh was removed, and dental implants were placed in position. Despite the extent of bone formation, the titanium remained inside the defect. The process of induced bone formation is a controlled response to highly concentrated levels of rhBMP-2.

Thus, the use of rhBMP-2, an osteoinductive agent, and allografts, which are osteoconductive, can enhance the amount and rate of bone formation [18].

3.5. GBR in Ridge Augmentation in Defects after Tooth Extraction

Vertical and horizontal alveolar ridge augmentation utilizing GBR has become a significant treatment option to provide optimal bone support for placement of osseointegrated dental implants. Vertical bone defects are more complicated to handle because of their high technical sensitivity [66].

In 1994, Simion et al. [67] performed a study in which the patients received conical Brånemark-type implants in sites requiring vertical augmentation. The implants protruded 4–7 mm from the bone crest, and titanium miniscrews were positioned distally to the implants, protruding 3–4 mm from the bone level. Both were covered with a titanium-reinforced membrane, and the flaps were sutured. After 9 months, the membranes were removed. There was a gain in bone height from 3–4 mm, and the implants were in direct contact with bone.

In a retrospective study in 1998, Tinti and Benfenati [68] evaluated the predictability of obtaining a vertical ridge augmentation around dental implants following GTR principles. The authors observed that when the clinical protocol was accurately followed, the possibility of clinical complications was reduced, and the results for achieving vertical ridge augmentation around implants were predictable.

Implants are often removed due to placement in unfavorable positions. Incorrect positioning of implants may result in biomechanical problems, loosening, and/or fracturing of the cover screws, implants, or implant collars in addition to occlusal discrepancies and compromised aesthetics and speech [69].

An ideal bone tissue volume is required for proper implant placement. Bone resorption will occur secondary to tooth extraction, mainly when multiple teeth are extracted at the same time. Among various ridge

expansion techniques, GTR for the treatment of ridge deficiencies is considered a safe and predictable treatment modality [70, 71].

Using this method, Toscano et al. [69] analyzed 73 consecutively treated lateral ridge augmentations that used composite material of demineralized freeze-dried allografts, mineralized cortical cancellous chips, and a biologically degradable thermoplastic carrier combined with a resorbable membrane for GBR in partial or completely edentate patients. An incision was made to the palate or lingual of the treatment site and was extended at least one tooth beyond in the mesial and distal directions. The periosteal was then released to allow for tension-free closure of the flap over the membrane and graft. Measurements of the pre-augmentation ridge width were made, and the bone defect was frayed to enhance revascularization of the site. The membrane was trimmed to fit the site and was applied to the thermoplastic composite graft. The graft was covered with the pretrimmed resorbable collagen membrane, and tension-free closure was performed. All cases were allowed to heal for a minimum of 6 months before implants were placed, and a second measurement was performed close to where the first measurement was made.

Researchers observed that the average presurgical ridge width was 4 mm. During stage I implant placement, an average ridge width postaugmentation of 7.5 mm was achieved, and the average gain in horizontal ridge width was 3.5 mm (range, 3–6 mm).

In 2012, Kao et al. [66] used a GBR technique and obtained satisfactory results for the edentulous area with severe bone loss. The patient suffered from advanced periodontitis of teeth 36, 37, and 47. Periapical radiographs revealed severe alveolar bone loss around teeth 36 and 37, which were subsequently extracted.

The GBR procedure was then performed. The flaps were elevated to expose the atrophic ridge and the immature healing sockets, and evident vertical and horizontal bone defects were found. The bleeding process was promoted, and two tenting screws were positioned. Autogenous bone was harvested from the mandibular ramus and mixed with FDBA particles. The Ti-reinforced e-PTFE membrane (TR6Y) was shaped for perfect adaptation and fixed to the lingual and buccal regions. The soft tissue was

secured with nonresorbable horizontal mattress sutures and interrupted sutures. Six months later, the second surgery was performed to remove the nonresorbable membrane, and the site revealed regenerated hard tissue covering the surface of the tenting screws.

The authors concluded that GBR with the Ti-reinforced e-PTFE membrane, tenting screws, and bone grafts offered predictable functional reconstruction of large vertical defects [66].

3.6. Studies in Animals

Kostopoulos et al. [72] conducted an interesting study in rats that has had a major clinical impact on cranial and maxillofacial surgery. They created bone increases equivalent to 5–6 times the original width of the mandibular ramus. The study involved exposure of the mandibular ramus; on the test side, the periosteum was left covering the lateral surface of the ramus, and on the other side (control), the periosteum was elevated together with the flap. A Teflon capsule was then placed to face the periosteum or the bone surface with the open part before the closure of the wound. At 120 days, the mean amount of bone obtained in the test specimens was 56%, reaching up to 52% in controls. These findings indicated that a secluded space created by an occlusive barrier adjacent to existing bone or periosteum may be filled with bone tissue.

This enabled GBR to be performed as an alternative method when it was necessary to obtain bone autografts from patients without sites having adequate amounts of donor bone.

In 1995, Bosch et al. [73] aimed to investigate the amount of bone formed in defects created experimentally in bone parietal. They protected the bone with one or two e-PTFE membranes in 29 Wistar rats, which were divided into two groups. In the double membrane group, the left experimental bone defect was protected by an outer e-PTFE membrane under the periosteum and parietal muscles and an inner membrane between the dura mater and the parietal bone. In the single membrane group, only the outer membrane was placed.

From this analysis, the authors observed that in the majority of specimens in the group in which only one membrane was used, the experimental site did not regenerate. In contrast, in the group with the double membrane, the majority of experimental defects had complete closure of the bone. These data demonstrated the importance of the separation of bone tissue and muscle tissue to obtain correct regeneration.

In 1997, Hurzeler et al. [74] extracted the maxillary canines and lateral incisors from eight rhesus monkeys. After a healing period and soft tissue expansion, implants were placed in the atrophic ridge on each side in such a way that its coronal 4–5 mm remained circumferentially exposed above the bone level. Two types of membranes were tested for implantation: 1) a bioresorbable barrier made of D,L-lactid-co-trimethylencarbonate (poly membrane) and 2) a nonresorbable e-PTFE barrier.

The results showed that the mean bone-to-implant contact length fraction was 32% of the total implant length with the polymembrane and 58% with e-PTFE, which exhibited significantly greater bone filling capacity than the polymembrane. Histologic observations of polymembrane specimens demonstrated a moderate inflammatory reaction related to the degradation and resorption products of the barrier. In conclusion, the nonresorbable e-PTFE GBR barrier was found to be superior to the bioresorbable barriers tested in the present investigation.

Li et al. [75] investigated the effects of guided bony regeneration using collagen membranes for sinus augmentation in the first maxillary molars of 18 adult female beagle dogs. After extraction of teeth, the sinus floors were lifted, and the implants were placed. A combination of autografts and Bio-Oss was used as a grafting material. The collagen membrane was folded at the lateral osteotomy window, at the apex of the implants, and at a certain part of the palatal bone. As a control group, the opposite site received a collagen membrane covering the osteotomy window. From the results, the authors observed grafted materials without resorption or subsidence on the experimental side, with new bone formation at the apex of the implants. On the control side, the grafted materials were shifted and absorbed. Histological examination showed increased formation of new bone in the experimental group, and this new bone matured over time. This study

showed that the presence of the membrane facilitated bone regeneration on the apical surface of the implants and that the only sinus membrane could not effectively stimulate formation of new bone in sinus augmentation.

Many materials have been studied for bone regeneration. The best results are found with utilization fresh autogenous materials due to its capacity for osteoinduction and osteoconduction. However, there are some disadvantages, as already discussed, such as pain and edema caused by additional injury to the donor site, the limited volume of bone tissue that can be harvested, and high graft-fragment resorption [76, 77]. Thus, other materials, such as allogeneic bone graft materials, which do not have bone regenerative capacity but do not require an additional donor site, have been used for bone grafting.

Using this method, Ahn et al. [78] evaluated bone formation after the use of allogeneic bone, with or without utilization of a membrane, in rats. The materials used in the experiment were the allograft Tutoplast Spongiosa Microchip, mineralized cancellous bone allografts (MCBAs), and absorbable membranes made from human pericardium and collagen. A 2-cm incision was made from the occipital bone to the frontal bone, and subcutaneous tissues were elevated together with the periosteum. A full-thickness defect area approximately 8 mm in diameter was then created. The control group was sutured without addition of graft material or the membrane. In group 1, the Tutoplast was transplanted into the area and sutured in place by closing the periosteum. In experimental group 2, the Tutoplast was transplanted into the defect area, covered with pericardium attached tightly to graft materials, and sutured by closing the periosteum.

In the control group, most areas were filled with loose connective tissue. In group 1 (allogeneic bone), bone from new bony spurs and host bone were observed on the edge of the defect area, with abundant proliferation of blood vessels and features of active bone formation. All animals showed immature bone formation in the center of the defect area and exhibited delayed bone fusion. In group 2 (allogeneic bone and absorbable barrier membrane graft), within 6 weeks, the membranes were partially absorbed, and numerous inflammatory cells were present. At week 12, the membrane boundary had disappeared in several areas,

indicating that membrane absorption had progressed substantially, and the center defect area was connected with the rim area and proliferated continuously. A bone-filling pattern was observed at the center of the defect area, and connective tissues were hardly detected.

Utilization of membranes is essential for osteoinductive regeneration. Membranes that are not degradable require a second surgery, which can be harmful to growing granular tissue. Thus, the use of absorbable membranes is recommended. As described by Ahn et al. [78], membranes inhibit connective tissue invasion, thereby facilitating new bone formation. Inflammation that occurs around the absorbable collagen barrier membrane is associated with degradation, as observed by Ahn et al. [78], and inflammatory cell infiltration was found to be restricted to the membrane rim and to not significantly affect bone regeneration [78].

Theoretically, the ideal bone substitute should have osteoconductive characteristics by acting as an intermediate-phase scaffold to give support during bone healing and osteoinductive characteristics by working as a guide to stimulate bone growth before being gradually replaced by the newly formed bone [79].

Deproteinized bovine bone mineral (DBBM) is an inorganic bovine bone derivative with a mineral content comparable to that of human bone, allowing integration of the material into bone. In addition to osteoconductive mechanisms, studies have suggested that this material may also trigger osteoinductive mechanisms [79].

Bioactive ions can also have favorable effects on the bone response during healing. Calcium sulfate (CaS) is a common bone substitute that is highly biocompatible and osteoconductive and undergoes virtually complete resorption *in vivo* [79, 80].

Turri and Danlin [79] evaluated the potential biological differences of a synthetic bone substitute material characterized by a high dissolution rate and release of high concentrations of ions in the wound area (CaS) with an osteoconductive material (DBBM) with a barrier membrane acting as control, in the GBR setting.

The CaS and DBBM particles were placed in defects created in the edentulous space between the incisors and molars in the maxilla of rabbits,

with or without placement of a membrane. After 2 weeks, total resorption of the CaS material was observed, regardless of the presence of a barrier membrane, and minor resorption was observed for the DBBM particles. Because of deproteinization, bone resorption was blocked by osteoclasts [81]. The CaS group showed more bone regeneration that the DBBM group. Moreover, the addition of an ECM membrane had substantial effects on bone regeneration owing to stimulation of angiogenesis in the early healing process [79].

4. GTR IN ORAL SOFT TISSUE

Oral mucosal defects are often an inevitable consequence of tumor excision. Recently, great progress has been made in reconstructive surgery for large soft-tissue defects in the oral cavity, resulting in increased survival rates in patients [82].

In small oral tissue defects, the use of palatal or buccal mucosal autografts or muscle may be appropriate [82, 83]. However, for reconstruction of large soft-tissue defects, cutaneous or myocutaneous flaps are needed [82].

Autologous skin grafts have been employed in animal and human studies; however, these grafts seem to maintain their original characteristics, such as hair growth, and are associated with donor-site morbidity [83]. Moreover, skin flaps are less flexible than the mucosa of the oral cavity and are inadequate for restoration of oral functions, such as speech, mastication, or swallowing [82]. Additionally, the use of skin flaps is associated with problems of cosmetic appearance. For example, the color and texture of the skin are not completely consistent with the tissues of the oral cavity [84]. Scar formation at the donor site may also occur, often necessitating secondary operations [82]. Thus, it is advisable to reconstruct intra-oral soft-tissue defects with soft tissues from the mucosa.

Wada et al. [82] described a procedure called biological-guided mucosa regeneration (BGMR). Based on the results of animal experiments and epithelialization of the grafted myofascia in the oral cavity, various

myofascial graft materials have been applied for reconstruction of intra-oral soft-tissue defects, thereby restoring nearly normal mucosa. This mucosal tissue shows good mobility of the intra-oral organs, as opposed to the more rigid and less flexible skin tissue.

The authors grafted myofascial flaps without skin in patients with oral defects caused by tumor resection in the tongue, buccal mucosal, lower gingival, floor of the mouth, and/or oropharynx.

The myofascial flap was tunneled up and inserted into defect in such a way that the fascial surface was oriented to face the oral cavity, and the myofascial flap was tightly sutured to the surrounding incised mucosal margin, resulting in a yellowish fibrin membrane on the surface.

Based on this clinical experience, using platysma myofascial flaps or pectoralis major myofascial flaps, the authors observed favorable progression of mucosal regeneration, with gradual epithelialization along the immature granulated myofascial and without clinical evidence of infection on the bare granulated myofascial. This method was useful for reconstruction of the inferior half of the oral cavity, including the oropharynx.

In summary, the authors presented a procedure for myofascial grafting that served as an alternative for the reconstruction of intra-oral soft tissue defects based on GTR principles.

Among the biomaterials employed by maxillofacial surgeons, those derived from animals are widely used; for example, catgut tissues are still popular. Collagen materials are also commonly used and are available in various forms, including laminates, sheets, fabrics, gels, and powders, and have been used as dressings for ulcers and burns and for induction of bone formation [84].

Rastogi et al. [84] utilized xenogenous, crosslinked collagen sheets as a cover for wounds in the oral cavity after excision of premalignant lesions, benign lesions, reactive proliferation, and incisional biopsy wounds in 60 patients. The collagen membrane showed good conformability, granulation, and epithelialization in most cases.

Wounds that are left uncovered in the oral cavity are prone to infection. Grafted wounds are known to heal faster than open wounds. The

best solution may be mucosal grafts; however, materials for these grafts are not sufficiently abundant. Although skin grafts may be the next-best solution, the disadvantages of these grafts have already been discussed.

In the study by Rastogi et al. [84], the collagen membranes slowly underwent collagenolysis and were sloughed off over time, allowing changes to occur in granulation tissues, which appeared to be clinically healthy and uniform. The adherence of the collagen membrane may be a result of the interaction between fibrin and collagen, but was most likely a result of fibrovascular ingrowth into the collagen membrane.

The collagen membrane exhibited minimal growth of granulation tissue and no major infection, thus supporting its use as a temporary biological dressing material in the oral cavity devoid of the mucous membrane. Accordingly, this material is an alternative to autologous grafts and a satisfactory addition to the armamentarium of oral surgeons as an excellent wound graft material [84].

5. FINAL CONSIDERATIONS

Based on the limited capacity of current strategies to reproduce tissues damaged by trauma, disease, anatomical or congenital defects, or tumors, surgeons have attempted to identify alternatives to tissue engineering approaches. Thus, tissue engineering strategies have been developed to facilitate reproduction of various body tissues.

However, when considering the craniofacial region, these approaches are more complex and challenging.

Today, GTR, a subfield within tissue engineering, is a reality and has been shown to be a very promising approach. The use of barriers to protect the tissue and select desirable cells to facilitate tissue regeneration, the presence of scaffolds, the use of growth factors, and the biocompatibility of tissue grafts have made the development of effective grafts that can restore lost tissue more achievable.

In dentistry, the utilization of GTR has been reported in surgery for maxillary sinus floor elevation, ridge augmentation for implant planning,

correction of OAC, reconstruction of defects after tumor resection, and treatment of alveolar cleft, among other applications.

Although GTR still needs to be more established in osseous tissues, this method, similar to GBR, should also be applicable in other tissues, including the oral mucosa, thereby contributing to successful tissue engineering.

Advances in GTR have expanded the range of treatment possibilities, allowing us to achieve the regeneration of ideal, well-formed, functional tissues; this approach should be studied in great detail in order to improve the potential success and applications of GTR-based therapies.

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EDITOR CONTACT INFORMATION

Luciano Tavares Angelo Cintra, DDs, PhD
Associate Proessor
Department of Endodontics
Dental School, São Paulo State University,
Araçatuba, SP, Brazil
Email: lucianocintra@foa.unesp.br

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