Periodontal regeneration

A project submitted to The College of Dentistry, University of Babylon, Department of periodontics in partial Fulfillment for the Bachelor of Dental surgery

SUPERVISED BY:

DR.DOAA NEAMAH MSEER

B.D.S, M.SC. PERIODONTICS

PREPARED BY:

Abdullah Saeed **Zahraa Fadhil**

Alaa jasim **Sara qasim**

Ahmed Mohammed

Essentials in Periodontal Regeneration

INTRODUCTION:

Progressive periodontitis can lead to tooth loss through the destruction of its attachment apparatus. When continued function necessitates additional periodontal support, optimal treatment should include not only periodontal infection control but also regeneration of the lost periodontium. Despite conclusive evidence that some regeneration may occur following regenerative procedures[1-2] , complete regeneration is an unrealistic goal. Osseous grafting and guided tissue regeneration (GTR) are considered as two of the most successful methods for reestablishment of periodontal tissues[2-3] . However, other treatment modalities have also shown promise in terms of improving clinical conditions and demonstrating significant bone fill. Periodontal regeneration is defined as reestablishment of the lost supporting tissues including alveolar bone, cementum, and PDL.

New connective tissue attachment is described as formation of new cementum with inserting collagen fibers in association with a root

surface that has been deprived of its PDL[4] . Bone fill is the clinical restoration of bone tissue in a previously treated periodontal defect .

Guided cell repopulation or guided tissue regeneration (GTR) are procedures designed to manipulate the cells that are involved in wound healing which finally lead to regeneration[4].

Regenerative Surgical Techniques (Flap procedures)

Regenerative periodontics can be divided into two major categories: non-graft-associated new attachment and graft-associated new attachment. A number of techniques have combined both procedures. These methods can be performed with and without flaps, but in most cases exposure of the area is preferable[5].

In non-graft associated regenerative procedures, reconstruction of periodontal tissues without using grafts is possible only in meticulously treated three wall defects (intrabony defects) and in periodontal and endodontal [5]urgical access procedures. Remodeling of two and three wall angular bone defects following a modified Wildman

flap requires careful curettage of the bone defect and proper root debridement[6-7] . "Modified flap operation" is basically an

access flap for proper root debridement. Bone regeneration in intrabony defects is considered as one of the major advantages of this technique [8]. "Coronally positioned flaps" have been used in

the treatment of mandibular class II furcation defects. In this technique the flap margin is positioned away from the furcation and

remains in that location during the early stages of healing[9].

Previous studies have shown vertical and horizontal bone fill in class II mandibular furcation defects[9]

In Graft associated regenerative procedures, graft materials are used in conjunction with flap procedures to stimulate periodontal regeneration. These materials can be classified into four types: autogenous, allogenic, xenogenic and alloplastic[10]

Autogenous bone grafts, Extra oral:

Autogenous iliac cancellous bone and marrow have been shown to possess a high degree of osteogenic potential. Numerous case reports have demonstrated successful bone fill after application of these materials in furcations, dehiscences, and intraosseous defects of various morphologies[11-12] . Iliac grafts can be used as either fresh or frozen. Root resorption has been reported as a complication of fresh grafting techniques[13-14] , which has led to the limited use of these materials in clinical practice

Autogenous bone grafts, intra oral:

Intraoral cancellous bone and marrow grafts are usually obtained from the maxillary tuberosity or a healing extraction site and are used

as cortical bone chips[15] , osseous coagulum or bone blend type grafts[16] . Some authors have reported the presence of a long junctional epithelium between the regenerated alveolar bone and root surface[17-18] . Thus, the presence of clinical bone fill does not necessarily indicate periodontal regeneration .

Allogenic bone grafts:

Several types of bone allografts exist such as iliac cancellous bone and marrow, freeze-dried bone allografts, and decalcified freeze-dried bone allografts. Frozen and radiation-sterilized iliac crest allografts have both been used in different studies. Freeze-drying has been shown to markedly reduce the antigenicity of allografts [19]. Intraosseous defects in juvenile periodontitis have been successfully treated with a combination of freeze-dried bone allografts and tetracycline[20-21] . According to Mellonig et al[22] , bone demineralization in 0.6N HCl followed by freeze drying can significantly increase the

osteogenic potential of allografts, assumably through bone morphogenic proteins (BMPs)[22-23].

A recent study has indicated that mineralized human cancellous allograft with or without collagen membrane, significantly improved bone fill in mandibular class II furcation defects[24]

Xenografts:

Xenogenic materials have also been used for grafting around periodontal defects. These grafting materials are also referred to as anorganic bone, probably because all cells and proteinaceous material are removed during processing. Consequently an inert absorbable bone scaffold is left behind upon which revascularization, osteoblast migration, and woven bone formation can take place [25]. Human histologic studies have reported signs of periodontal regeneration in teeth treated with bovine-derived xenografts[26-27] . Xenografts have shown superior results when used in combination with guided bone regeneration (GBR) methods around implants and in sinus lift and ridge augmentation procedures[28-29]

A recent study found that porcine bonederived biomaterials can be successfully used in humans for maxillary sinus augmentation prior to implant placement[30]

Alloplasts:

Alloplasts are synthetic, inorganic, biocompatible bone substitutes which promote bone healing. There are presently six types of alloplasitc materials used in clinical practice which are as follows: nonporous hydroxyapatite (nonresorbable), porous hydroxyapatite or

replamineform (nonresorbable), hydroxyapatite cement, beta tricalcium phosphate (resorbable), HTR (a calcium layered polymer of

polymethylmethacrylate and hydroxyethylmethacrylate, nonresobable) and bioactive glass.

Several studies have demonstrated superior results in defects grafted with nonporous[31] and porous hydroxyapatitie[32] , HTR[33] and beta tricalcium phosphate [34] as compared to those treated without the use of grafts.

While clinical findings appear promising, histologically the grafts tend to be encapsulated by connective tissue with minimal or no bone formation [35]. Microscopic studies have found limited new bone in proximity to the implanted materials[36] .

There is histologic evidence suggesting that a limited amount of regeneration may occur ,following HTR grafts[37] Poehling et al[38] , indicated that MD05،

consisting of β-TCP coated with recombinant human growth/differentiation factor-5 (rh GDF-5), achieved superior bone regeneration

compared to conventional materials. It was concluded that MD05 may be a suitable new bone substitute for application in dental and

maxillofacial surgeries. Bioactive glass (BG) is made from calcium salts, phosphate, sodium salts, and silicon glass particles. This silicon layer stimulates the formation of a hydroxyl carbonate-apatite layer onto which osteoblasts can proliferate and produce bone[39].

A recent animal study investigated the effects of bioactive glass within a titanium cap. New bone was found to be generated at an early stage following utilization of BG for bone augmentation[40].

Mengel et al[41] studied the long term effectiveness of a bioabsorbable membrane and a bioactive glass in the treatment of intrabony defects in patients with generalized aggressive periodontitis. The results indicated significant improvements in

probing depth (PD) and clinical attachment level (CAL) after 5 years with both regenerative materials. Radiographically, the bioactive glass group revealed superior bone fill .

Guided Tissue Regeneration (GTR):

The term “Guided Tissue Regeneration (GTR)” was given by Gottlow in 1986. The 1996 World Workshop in Periodontics defined GTR as “procedures attempting to regenerate lost periodontal structures through differential tissue responses. Barriers are employed in the hope of excluding epithelium and gingival corium from the root surface in the belief that they interfere with regeneration”. The rationale behind using GTR membranes is to exclude epithelium and gingival connective tissue, maintain space between the defect and tooth, and stabilize the clot. According to Melcher hypothesis [39] certain cell populations residing in the periodontium have the potential to create new cementum, alveolar bone and periodontal ligament, when they have provided the opportunity to populate the periodontal wound. Melcher hypothesis was experimentally established and histologically verified by Karring et al. [40].

They have shown that such conditions arise when gingival epithelial cells or fibroblasts are excluded from the wound space and periodontal ligament cells are allowed to migrate and populate the wound space. The necessity for exclusion of epithelium and connective tissue cells of the gingiva from wound led to development of periodontal devices know as barriers or membranes for guided tissue regeneration. The first GTR membrane used in the periodontal surgery was cellulose acetate laboratory filter paper by Nyman et al. [41] in 1982. This barrier lacked several characteristics necessary for guided tissue regeneration

Characteristics of GTR membranes:

Characteristics or design criteria for GTR membranes have been proposed by Scantlebury [42] in 1993 are biocompatibility, cell exclusion, space maintenance, tissue integration and ease of use. An additional characteristic, biological activity should be considered for future regeneration devices. Black [43] defined biomaterial as a nonviable material used in medical device, intended to interact with biological systems. Any device introduced into the body to address a particular need has to fulfill two major requirements, safety and efficacy. Safety is addressed through a wide selection of in vitro and in vivo assays, designed to address specific aspects of biocompatibility. Biocompatibility is defined by Williams [44] as the ability of a material to perform with an appropriate host response in a specific situation, which means that neither the material adversely and significantly affects the body nor the physiological tissue environment adversely and significantly affects the material. Ten assays used to evaluate biocompatibility are cell culture cytotoxicity, skin irritation, subcutaneous implantation, blood compatibility, hemolysis, carcinogenesis, mutagenicity, pyrogenicity, sensitization, and short-and long –term histological tissue reaction. Cell exclusion requires the membrane to separate gingival flap from the maturing fibrin clot in the wound space. No experiments specifically addressing this aspect of GTR membrane. Space maintenance for regeneration requires mechanical properties

and/or structural features allow membrane to withstand the force of flap (tissue tension) or occlusion and prevent collapse of soft tissue and elimination or reduction of wound space. Tissue integration dictates the incorporation of structural elements in the membrane to promote tissue ingrowth which concurrently

achieve cell exclusion. Easy to use means membrane should be clinically manageable i.e. competent clinician can use membrane without undue difficulty

Membrane can be non-absorbable or absorbable:

Non absorbable barriers:

Non-absorbable membranes maintain their structural integrity for as long as they are left in the tissues. The function of membrane is temporary and once function is completed, there is no longer any need for it to remain in place. Although tissue integrity of membrane can be achieved, membrane is susceptible to risk of latent or post-surgery bacterial contamination which indicates removal of membrane to be in the best interest of the patient. POLYTETRAFLUOROETHYLENE (PTFE) is a non-absorbable membrane having formula (-CF2-CF2-)n hence, it is a fluorocarbon polymer. Solid/dense PTFE (dPTFE : TefGen-FD) [45] is non-porous, does not allow tissue ingrowth and does

not elicit foreign –body reaction. Expanded PTFE (ePTFE : Gore-Tex) is porous microstructure of solid nodes and fibrils, allows tissue ingrowth, exhibits minimal inflammatory tissue reaction, formed when dPTFE subjected a tensile stress and used as a vascular graft material. ePTFE membrane has been modified by incorporation of titanium reinforcements, set between two layers of ePTFE, resulting in identical surface properties and better mechanical strength. Gore-Tex periodontal membrane has two structural designs, a coronal open microstructure collar, and a cell- occlusive apical portion. Collar promotes tissue ingrowth, support wound stability and inhibit epithelial migration is 1 mm thick, low density (0.2 g/ml)

and 90 % porous (100-300 μm between nodes). Apical portion, serving as a space provider for regeneration as well as a barrier towards the gingival flap is 0.15 mm thick, higher density (1.5 g/ml) and 30 % porous (<8 μm between nodes). Gottlow et al. [46] in 1986 showed new attachment formation in human periodontium by ePTFE membrane in 3 months. Cortellini et al. [47] in 1993 showed periodontal regeneration of human intrabony defects by ePTFE membrane in 6 months. Murphy [48] in 1995 showed minor post-operative healing complications of ePTFE membrane as pain, purulence, swelling and tissue sloughing with an incidence slightly higher than conventional periodontal surgery. Other non-absorbable membranes are RUBBER DAM, RESIN/GLASS-ionomer barrier and

COMPOSITE barrier. Rubber dam and resin/glass-ionomer barriers do not fulfill design criteria for GTR membrane. A composite membrane (BioBrane) [49] is made from knitted nylon fabric mechanically bonded onto a semipermeable silicone membrane and coated with collagen peptides have been evaluated in animals but gives mixed results in terms of regenerative potential.

Absorbable barriers:

Absorbable barriers are biodegradable, hence do not require their removal which reduce patient discomfort and eliminate surgery related complications. Absorbable membrane's disintegration process starts immediately after placement in the surgical site and their rate of disintegration vary from individual to individual, hence there is no control over length of application. Minabe [50] in 1991 reported that absorbable barriers should maintain their in vivo structure at least 4 weeks for biological rationale of GTR. Due to their biodegradable nature absorbable barriers elicit tissue reactions which influence wound healing and regeneration. Absorbable barriers can be natural or synthetic.

Bone grafting

The types of grafts that were utilized included autogenous grafts (derived from the same individual), allogenic grafts (derived from a different member of the same species), xenografts (derived from different species) and alloplastic materials (synthetic products). The clinical performance of bone grafting procedures in the regeneration of intrabony andfurcation defects has been comprehensively assessed in a recent systematic review.5

Factors affecting the clinical outcome of regenerative procedures

The clinical outcomes using regenerative therapy vary extensively between different studies, with some reports describing average attachment gains as high as 5.3mm,57while others reporting far inferior outcomes of as little as 0.6 mm.58 Therefore, it is important to consider the factors that influence the clinical outcomes in regenerative procedures. These factors can broadly be divided into factors associated with the patient, tooth, defect and surgical technique.

Patient factors

Patient related factors which influence the success of regenerative therapy include oral hygiene and smoking. It is important that the patient's periodontitis is successfully treated prior to the commencement of periodontal regeneration. As part of this treatment, it is essential that the patient achieves a high level of self- performed oral hygiene as superior results have been obtained in patients with optimal compared to less ideal oral hygiene.59,60 Furthermore, cigarette smoking has also been shown to negatively affect the clinical outcomes following regenerative therapy.59Other patient related factors such as genetics, age, various systemic conditions and stress have been adversely affect the outcome of regenerative proposed to procedures, but since there is no evidence to support these assumptions, they should not influence the decision to proceed with regenerative therapy unless there is a contraindication for elective surgery (e.g., uncontrolled diabetes).

Tooth factors

Tooth related factors that may affect the treatment response to periodontal regenerative therapy include the endodontic status of the tooth and hypermobility. It has been reported that endodontically compromised or inadequately treated teeth respond less favourably to periodontal treatment.61,62

However, it has also been shown that adequately performed root canal therapy does not affect the success of regenerative therapy.63 Tooth mobility has reported to be negatively and dose dependently associated with inferior outcomes following periodontal regeneration.64 although it has been shown that teeth with baseline horizontal mobility of less than 1 mm can be successfully treated. 65

Defect factors

The nature of the periodontal defect can have a significant impact on the success of periodontal regeneration. Generally, it is recognized that currently available clinical techniques are limited to the treatment of intrabony and Class II furcation defects, and there is no evidence that suprabony (horizontal), interdental craters, supracrestal components of intrabony defects or Class III furcations can be predictably regenerated. The anatomy of the periodontal defect can influence the outcome of the regenerative procedure, with superior regeneration associated with deep, narrow defects, and increased number of residual bony walls.60,66This is particularly the case with non-supported defects, and it has been shown that the adjunctive use of a bone replacement graft for its ‘‘supportive’’ and ‘‘space-making’’ properties can over come the detrimental effects of adverse intrabony defect morphology.67

Surgical factors

The realization that membrane exposure adversely affects the outcome of regenerative techniques, especially when associated with non-resorbable materials, led to the development of modified surgical techniques specifically designed to preservt interdental tissues.68,69.

Indeed, these clinical procedures, which utilize papilla preservation flap designs and suturing techniques that provide tissue stability and allow primary closure of interdental tissues, have been shown to be associated with superior regenerative outcomes.70,71.

CONCLUSIONS

Over the past 25 years, periodontal regeneration has been the focus of considerable laboratory and clinical research. Indeed, numerous randomized controlled clinical trials have been carried out in order to assess the clinical effectiveness of several surgical techniques aimed at achieving periodontal regeneration. From the evidence available in the literature, the following conclusions can be reached:

(1) Currently, there are two well-documented clinical techniques, GTR and EMD, which can be utilized for the regeneration of intrabony and Class II mandibular furcation periodontal defects.

(2) In cases where additional support and space-making requirements are necessary, both of these procedures can be combined with a bone replacement graft.

(3) There is no evidence that the combined use of GTR and EMD results in superior clinical results compared to the use of each material in isolation.

(4) Great variability in clinical outcomes has been reported in relation to the use of both EMD and GTR, and these procedures can be generally considered to be unpredictable. Careful case selection and treatment planning, including consideration of patient, tooth, site and surgical factors, is required in order to optimize the outcomes of treatment.

(5) There is limited data available for the clinical effectiveness of the commercially manufactured products PDGF/b-TCP and P-15/ABM, and further studies are required to test the clinical performance of these two products.

(6) The use of PRP for periodontal regeneration has yielded contradictory results and further studies are required to determine the optimal conditions and methods of preparation.

Regulatory Mechanisms of Periodontal Regeneration

periodontal ligament is located between the ce-mentum and the alveolar bone. The width of the peri-odontal ligament ranges from 0.15 to 0.38 mm,
whichnarrows with age, and has a close relationship withfu nctional states of the tooth [72] .

Peri-odontal ligament fibroblasts are a heterogeneous pop-ulation of cells that are involved in the normal main-tenance, repair, and regeneration of both the ligamentand adjacent hard tissues [73] .

The major functions of the periodontal ligament include tooth support, proprioception, nutrition, homeostasis, and repair. Among these, the homeostaticand repair functions are the most significant with respect to periodontal therapy, transplantation and/orreplantation of teeth, and orthodontic tooth movement.These functions involve control over cellular activitiessuch as proliferation, synthesis and resorption, collagen turnover, resorption and repair of the root cementum, and remodeling of the alveolar bone proper [74].

It has been confirmed that the periodontal ligamentis essential for osteogenesis and cementogenesis inperiodontal tissues and that proliferative cells, such asundifferentiated mesenchymal cells, which are inducedby injury stimulation (including tooth extraction), candifferentiate into osteoblasts or cementoblasts. On theother hand, it has been reported that there is a mechanism suppressing the synthesizing function of boneand cementum that maintains a fixed space in theperiodontal ligament [75] .

This implies a complicated mechanism,consisting of systems both promoting and suppressingthe bone or cementum

formation in order to accomplishhomeostasis and repair in the periodontal ligament.Analogous results on periodontal regeneration havebeen reported by a large number of researchers [76].

It has recently been noted that growth and differen-tiation factors regulate cellular function in the peri-odontal ligament during regeneration and homeosta-sis. Growth factors are proteins that may act locally orsystemically to affect the growth and function of cells invarious manners. They may act in an autocrine or aparac rine fashion and control the growth of cells avail-able to produce a specific tissue. Growth factors, includ-ing the platelet-derived growth factor, insulin-likegrowth factor, transforming growth factor, basic fibro-blast growth factor, and bone morphogenetic protein,may regulate the metabolism of a particular cell typeincluding the production of an extracellular matrixcomp onentifferentiation factors regulate the pheno- typic state of cells , causing undifferentiated mesenchy-mal cells (precursor cells) to differentiate into osteo- blasts (fully functional mature cells) [77].

Cell-surface proteoglycans are nownto participate in several biological functions such ascel

l-cell and cell-matrix interactions, in cell dhesion,in binding to various growth factors as co-receptors,and in repair [78].

The importance of enamel matrix proteins in thedevelopment of cementum and periodontal tissues hasbeen demonstrated. A number of studies now indicatethat cells of Hertwig's epithelial root sheath have as ecretory activity of enamel protein similar to that ofameloblasts. Experiments in monkeys have shown thatit is possible to induce the formation of acellular cementum by applying porcine enamel matrix on a denuded root surface, thereby promoting periodontalregeneration [79].

Numerous clinical studies have proven that alveolarbone is synthesized after autogenous tooth transplantation and replantation [80].

In contrast, only afew investigations on the precise mechanisms havebeen reported with regard to the relationship betweentooth implantation or replantation and regeneration ofthe periodontal ligament [81].

Furthermore, it is well known that the homeostaticfunction of the periodontal ligament maintains afixedwidth following orthodontic tooth movement. However,a detailed explanation remains to be given why boneresorption occurs at the pressure side while bone pro-duction occurs at the tension side during orthodontictooth movement

The epithelial rests of Malassez represent the devel-opmental residues of Hertwig's epithelial root
sheathand are localized in the periodontal ligament tissuene ar the root cementum [82] .

Al-though the exact function(s) of the epithelial cell rest ofMalassez is still obscure, there is general consensusthat it is capable of reactive proliferation and mayparticipate in maintaining thefixed width of the peri-odontal ligament during its regeneration [83] .

This review describes the mechanism of tissue regen-eration and the homeostatic function of the periodontalligament. In particular, the regenerative capability
ofthe ligament tissue and the function of a variety ofgrowt

h factors and extracellular matrices that haverecently been characterized are discussed in relation toperiodontal regeneration. The biological mechanism in-volved in the maintenance of the periodontal
ligamentat afixed width is considered from the viewpoint o fperiodontal treatment, including tooth
transplantationor replantation and orthodontic tooth moveme nt. Ahypothetical mechanism for the regulation of regener-ation and homeostasis in the periodontal ligament ispresented.

Application of periodontal ligament cell sheet for periodontal regeneration

Objective: The ultimate goal of periodontal treatment is to regenerate the damaged periodontal support. Although periodontal ligament (PDL) cells are essential for periodontal regeneration, few studies have reported the transplantation of periodontal ligament cells to periodontal defects. We developed a new method to apply periodontal ligament cells as a sheet to the defect. The aim of this study was to investigate the periodontal healing after application of the periodontal ligament cell sheet in beagle dogs [84] .

Methods: Autologous periodontal ligament cells were obtained from extracted premolars of each beagle dog. Periodontal ligament cell sheets were fabricated using a temperature-responsive cell culture dish. Dehiscence defects were surgically created on the buccal surface of the mesial roots of bilateral mandibular first molars of each dog. In the experimental group (five defects), periodontal ligament cell sheet with reinforced hyaluronic acid carrier was applied to the defect. Only the hyaluronic acid carrier was applied to the contralateral side as a control (five defects). Eight weeks after surgery, the animals were sacrificed and decalcified specimens were prepared. Healing of the periodontal defects was evaluated histologically and histometrically

Results: No clinical signs of inflammation or recession of gingiva were observed in both experimental and control groups. In the experimental group, periodontal tissue healing with bone, periodontal ligament and cementum formation was observed in three out of five defects. In the control group, such periodontal tissue formation was not observed except in one defect. Histometric analysis revealed that the formation of new cementum in the experimental group was significantly higher than that in the control group.

Conclusion: The periodontal ligament cell sheet has a potential to regenerate periodontal tissue and may become a novel regenerative therapy.

Biological Mediator-associated new

Attachment and regeneration

Substances used to modify root surface:

1. Citric acid

2. Tetracycline

1-Citric acid

1. Produces 4 mm deep demineralized zone exposed collagen fibers

2. Eliminate endotoxins and bacteria

3. Early fibrin linkage prevents epithelium migration

The outcomes of controlled clinical trials have failed to show any

Improvements in clinical conditions compared to non–acid-treated controls.

Biomodificationof rootsurface

2-Tetracycline

- stimulates fibroblast attachment

- Suppresses epithelial cell attachment

And migration

- Remove amorphous surface layer

- Exposes dentinal tubules

-enamel matrix derivate (EMD)

- Marketed as Emdogain□

- Obtained from developing porcine teeth

- Induces acellular cementum formation

- The major (>95%) component of EMD is AMELOGENINS

- Extracellular Matrix Proteins purified acid extract of

Proteins from pig enamel matrix

- EMD alone or in combination with grafts has demonstrated

Its potential to effectively treat intraosseous defects and the

Clinical results appear to be stable long term .

Growth Factors for use in periodontal

Regeneration:-

-Bone morphogenetic proteins

(BMPs)

- Commercially available as recombinant human BMP-2

(rhBMP-2)

- Induced the differentiation of mesenchymal stem cells to

Become osteoblasts(bone forming cells)

PRP (Pletelet rich plasma) is a concentrated source of

Autologous platelets.

PRP is used to deliver growth factors in high concentrations to

The site of the bone defect or a region requiring augmentation.

Growth factors:-

Platelet derived growth factor (PDGF)

Transforming growth factor (TGF)

Platelet derived growth endothelial growth factor (PDEGF)

Platelet Rich Fibrin(PRF)

PRF was first developed in France by Choukron et al.

The advantages of PRF over PRP are its simplified

Preparation and lack of biochemical handling of the blood.

Assessment of periodontal wound healing:

Histological Methods

It is only through histological analysis can one define the nature of the reparative tissue . In periodontal reconstructive surgery, the goal is to achieve periodontal regeneration. Classically, experimental animal model systems are used whereby reference notches are placed at the base of bony defects or at the apical extent of calculus deposits. Periodontal regeneration is considered to have occurred when the newly formed functionally aligned periodontium is coronal to the apical extent of the notches. Reparative tissue response dominated may include long junctional epithelium, connective tissue adhesion, and root resorption associated with ankylosis. It should be noted that the healing may be predominated by periodontal regeneration; there may be localized areas of repair. Unfortunately, this approach cannot be used in human studies as it would be unethical to extract the treated tooth, especially when it responded positively to therapy. On rare circumstances, human histology is available if the tooth is to be extracted in conjunction with orthodontic or restorative therapy

Clinical Methods

Clinical methods to evaluate periodontal reconstruction consist of comparisons between pretreatment and posttreatment pocket probings and determinations of clinical gingival findings. The probe can be used to determine pocket depth, attachment level, and bone level . Clinical determinations of attachment level are more useful than probing pocket depths because the latter may change as a result of displacement of the gingival margin

Several studies have determined that the depth of penetration of a probe in a periodontal pocket varies according to the degree of inflammatory involvement of the tissues immediately beneath the bottom of the pocket . Therefore, even though the forces used may be standardized with pressure-sensitive probes, there is an inherent margin of error in this method that is difficult to overcome. Fowler et al71 have calculated this error to be 1.2 mm, but it is even greater when furcations are probed

Bone probing performed with the patient under anesthesia is not subject to this error and has been found to be as accurate as bone height measurements made on surgical reentry.

Measurements of the defect should be made before and after treatment from the same point within the defect and with the same angulation of the probe. This reproducibility of probe placement is difficult and may be facilitated in part by using a grooved stent to guide the introduction of the probe .

Preoperative and postoperative comparability of probing measurements that do not use this standardized method may be open to question.

Radiographic Methods

Radiographic evaluation of periodontal regeneration allows assessment of the bone tissue adjacent to the tooth. This technique also requires carefully standardized techniques for reproducible positioning of the film and the tube. Even with standardized techniques (see Chapter 31), the radiograph does not show the entire topography of the area before or after treatment. Furthermore, thin bone trabeculae may exist before treatment and go undetected radiographically because a certain minimal amount of mineralized tissue must be present to register on the radiograph. Several studies have demonstrated that radiographs, even those taken with standardized methods, are less reliable than clinical probing techniques. A comparative study of pretreatment bone levels and posttherapy bone fill with 12-month reentry bone measurements showed that linear radiographic analysis significantly underestimates pretreatment bone loss and posttreatment bone fill.

Studies with subtraction radiography have enhanced the usefulness of radiographic evaluation.A comparative study of linear measurement, computer-assisted densitometric image

analysis (CADIA), and a method combining the two reported that the linear-CADIA method offers the highest level of accuracy.

Surgical Reentry

The surgical reentry of a treated defect after a period of healing can provide a good view of the state of the bone crest that can be compared with the view taken during the initial surgical intervention and can also be subject to measurements . Models from impressions of the bone taken at the initial surgery and later at reentry can be used to assess the results of therapy

This method is very useful but has two shortcomings: It requires a frequently unnecessary second procedure, and it does not show the type of attachment that exists (i.e., new attachment or long junctional epithelium35).

Nanomaterials for Periodontal Tissue Regeneration: Progress, Challenges and Future Perspectives

The oral cavity plays a crucial role in essential body functions such as mastication, speech or deglutition, and its influence on facial aesthetics, especially of teeth, is undeniable. Periodontal tissue, including the alveolar bone, periodontal ligament (PDL), cementum and gingiva, is essential to maintain the integrity and stability of the teeth, absorb the chewing forces, and protect

against bacterial invasion and infection. There are a variety of factors causing periodontal tissue defects and threatening the patients' quality of life, such as caries, periodontitis, tumors, cysts and trauma [91]. Due to the complex functions of the oral tissue and the unique characteristics of the oral environment, it has always been a great challenge to reconstruct the periodontal tissue and restore its physical function.

For periodontal tissue reconstruction, traditional techniques based on allogenic grafts replacing the missing or damaged tissue from living donors or even cadavers are still used in dental and other medical fields [92]. For example, autologous and allogenic alveolar bone grafts are currently considered a gold standard to overcome bone atrophy, although in clinical settings the best results seem to be obtained with autologous bone [93]. However, these methods encounter limitations such as limited supply of graft tissue, donor site morbidity, graft failure, immunological rejection and lengthy hospitalization periods [94]. In addition, these techniques exhibit great difficulty in regenerating the cementum–ligament–bone complex. Furthermore, traditional dental materials such as composites and cement in macro and micro sizes are also widely applied in clinics. Despite their low cost, easy application and good biocompatibility, these materials also present several complications, such as degradation [95], cure shrinkage [96], stress fatigue [97], marginal microleakage [98] and high

susceptibility to microbial adhesion. Thus, there is a critical need for alternative techniques and materials.

In this respect, nanotechnology can provide an innovative alternative. Nanomaterials, in the range of 1–100 nanometers, have gained significant attention in regeneration medicine due to their unique optical, mechanical, magnetic, electronic and catalytic properties [99], which explain their high biocompatibility, permeability, tunability and immune evasion capability. Hence, they exhibit tremendous potential in tissue engineering [100], as anti-bacterial agents [101], for drug delivery [102], tissue repair [103] and functional imaging (such as MRI and CT) [104]. Recently, various types of nanomaterials, such as nanoparticles, nanocapsules, nanocomposites, nanofibers, nanotubes and nanosheets, have achieved satisfying outcomes and could therefore be used to reconstruct structures and restore the functions of oral tissues [105].

However, nanotechnology and nanomaterials also face great challenges that prevent them from advancing from the bench to the clinic. Firstly, there is a shortage of established protocols that allow their construction with the desired composition, structure parameters and physiochemical properties. It is difficult to modify or improve the behavior or performance of nanosystems in vivo because of the limited development of their

surface chemistry [106]. Second, the biosafety issues and adverse events of nanomaterials remain concerning. For instance, in vivo application of nanomaterials could induce immunologic reactions [107]. Moreover, nanomaterials have shown high permeability [108], and therefore their cytotoxicity can significantly increase as they can penetrate through physiological barriers and may accumulate in nontarget tissues [109]. Third, it is very difficult to control their biodegradation. Their biodistribution and pharmacokinetics largely depend on the size, shape and surface chemistry and homogeneous production of nanomaterials is still a challenge [110].

Moreover, when nanomaterials are applied for the local delivery of drugs, it is essential to control their biodegradation rate [111]. Although many papers emphasize their promising perspective in tissue regeneration, these challenges are rarely discussed.
This review focuses on the most recent publications regarding periodontal tissue regeneration with nanotechnology and nanomaterials . First, the current designs, structures and functions of nanomaterials are introduced and discussed. Secondly, the related factors that may interact with the behaviors and bioactivities of nanomaterials are summarized and elucidated. Lastly, the potential complications of nanomaterials are presented and some remarks for future

research are proposed in order to overcome the current limitations.

Discussion:

Alveolar Bone Regeneration

The alveolar bone is a specific type of bone tissue that forms the sockets where the teeth are supported and anchored to the jaws. The alveolar bone is constantly remodeling itself in response to various factors, such as chewing forces and hormonal changes. Under physiological conditions, this process involves the breakdown and replacement of old bone tissue by new bone tissue, which helps to maintain the integrity and strength of the bone. However, under pathological conditions, the breakdown of alveolar bone is spontaneously irreversible and can lead to tooth mobility and loss [112]. Most of the studies involving periodontal tissue regeneration focus on alveolar bone regeneration. Various techniques have been proposed to guide or control bone regeneration, such as bone grafting [113], guided tissue regeneration (GTR) [114] and platelet-rich plasma (PRP) therapy [115]. Although effective to a certain level, these techniques lack in repeatability and do not completely reconstruct the original periodontium [116,117]. Recently, the application of nanomaterials smaller than 100 nm on the above

techniques has shown promising results, since they have multiple advantages over traditional materials, such as versatility, biocompatibility, enhanced cellular interactions, improved tissue integration, controlled drug release and mechanical strength [118,119,120].

Among the included studies, the nanomaterials applied in alveolar bone regeneration are listed in Table 1. Nano-hydroxyapatite (nHA) [121,122,123,124,125,126,127,128,129] and collagen [130,131,132,133,134,135] are the most commonly investigated biomaterials for alveolar bone regeneration. Hydroxyapatite is a naturally occurring mineral that is the main component of bone tissue, and nHA has a higher surface area-to-volume ratio compared to conventional hydroxyapatite, making it more suitable as a substitute for bone graft [136]. Collagen is a crucial component of the extracellular matrix in many tissues, including alveolar bone. It provides structural support, enables cell–biomaterial interaction and increases cell adhesion, which helps regulate cell behavior, making it an attractive biomaterial for alveolar bone regeneration [137]. Both nHA and collagen have excellent osteoconductive properties, which can support the formation of new bone tissue by serving as a scaffold for bone growth, with good biocompatibility and mechanical properties.

Poly(lactic-co-glycolic acid) (PLGA) [133,134,138,139,140,141] and polycaprolactone (PCL) [

128,142,143,144,145] are biodegradable polymers that have been widely studied as materials for nanoparticles but also in other applications in tissue engineering and regenerative medicine. Both PLGA and PCL degrade over time into natural compounds that can be metabolized and eliminated through normal metabolic pathways. This facilitates their application in bone grafting, guided tissue regeneration and especially drug delivery. When engineered to release therapeutic agents, such as growth factors or antibiotics, they can provide sustained long-term therapeutic effects that can promote bone regeneration and prevent inflammation [146]. However, PLGA and PCL also present shortcomings.

For example, the mechanical strength of PLGA alone is inadequate for bone tissue regeneration, so PLGA often needs to be incorporated with other ceramic nanoparticles such as nHA, PCL and flourhydroxyapatite [147]. Moreover, although PCL displays adequate cell adhesion and good mechanical strength, its slow biodegradation, which takes more than 2 years, greatly exceeds the time required for new tissue formation [148], which can negatively impact the bone tissue regeneration process [149].

Gold nanoparticles (AuNPs) have attracted much attention as multifunctional contrast agents for computerized tomography (CT) due to their chemical inertness, versatile surface

functionalization and biocompatibility, high radiopacity and low cytotoxicity [150,151]. In addition, in vivo cell labeling and tracking using AuNPs with CT have become a cost-effective and time-efficient approach [152].

RATIONALE FOR REGENERATIVE PERIODONTAL THERAPY:

The main goal of periodontal therapy is to treat the infection caused by periodontal pathogenic biofilm and to arrest or slow down further attachment and bone loss, ultimately preventing tooth loss. Successful treatment is defined clinically by reduction of probing pocket depths (PPD), resolution in inflammation (i.e., resolution of suppuration and reduction of bleeding scores) along with the re-formation of a dento-gingival environment that allows effective oral hygiene measures. Ideally, these clinical improvements should be also accompanied by gain of clinical attachment level (CAL) and radiographic bone gain. There is ample evidence that in the great majority of cases, these goals can be achieved with the

first and second step of periodontal treatment consisting of patient motivation and instruction for successful removal of supragingival dental biofilm and control of risk factors known to be associated with the deterioration of periodontal status such as smoking, and diabetes (step one) followed by nonsurgical subgingival instrumentation (step two).1

However, in particular areas/defects, the endpoints of therapy defined as no periodontal pockets ≥4–5 mm with bleeding or residual probing depths ≥6 mm, are not always achieved following steps one and two. For such deep sites which persist after completion of steps 1 and 2, further treatment (i.e., the so-called step three) is needed, in order to reach the treatment endpoints, and thus enable the patients to be enrolled in a periodontal maintenance program (i.e., step 4) to prevent recurrence of the disease. Based on the individual case and defect, step 3 may consist of a surgical access (i.e., either conventional, resective, or regenerative) aiming to facilitate subgingival instrumentation, and to either resect or regenerate the residual soft and hard tissues to re-establish an environment favorable for proper supragingival biofilm control.

In the presence of deep intrabony (angular) defects and class II molar furcation involvements, resective surgery may lead to relevant clinical improvements by decreasing deep pockets to a more maintainable range, but the healing is accompanied by substantial loss of attachment and increase in soft tissue

recessions. Therefore, it is generally recommended that residual deep pockets associated with angular bony defects with an intrabony component ≥3 mm or deeper and class II mandibular and buccal maxillary furcations are rather treated by means of regenerative periodontal surgery than via a resective approach.

REFERENCES:

1. Cole RT, Crigger M, Bogle G, Egelberg J, Selvig KA. Connective tissue regeneration to periodontally diseased teeth. A histological study. J

Periodontal Res 1980 Jan;15(1):1-9

2. Bowers, GM, Chadroff B, Camevale R. Histological evaluation of new attachment apparatus formation in humans. Part I. J Periodontol 1989;60: 664-74.

3. Gottlow J, Nyman S, Lindhe J, Karring T Wennström J. New attachment formation in the human periodontium by guided tissue regeneration Case reports. J Clin Periodontol 1986 Jul;13(6.١٦-٦٠٤:)

4. American Academy of periodontology. Glossary of periodontic terms. 3rd edition .1992.

5. Fermin A. Carranza , Henry H. Takei, David L Cochran. Reconstructive periodontal surgery. In Fermin A. Carranza, Klokkevold, Henry . H.Takei Clinical Periodontology. Philadelphia: W.B.

Saunders; 2006; p. 968

6. Rosling B, Nyman S, Lindhe J. The effect of systematic plaque control on bone regeneration in infrabony pockets. J Clin Periodontol 1976 Feb;3(1):38-53

7. Polson AM, Heijl LC. Osseous repair in infrabony periodontal defects. J Clin Periodontol

١٩٧٨Feb;5(1):13-23

8. Kirkland O. The suppurative periodontal pus

pocket; its treatment by the modified flap

operation. J Am Dent Ass 1931: 18: 1462–1470.

9. Gantes B, Martin M, Garrett S, Egelberg J.

Treatment of periodontal furcation defects. (II.(

Bone regeneration in mandibular class II defects. J

Clin Periodontol 1988 Apr;15(4):232-9.

10. Jan Lindhe, Thorklid karring, Pierpaolo

cortellini. Regenerative periodontal therapy. In:

Jan Lindhe, Tharklid karring, Niklaus P. Lang.

Clinical periodontology and Implant Dentistry.
New Jersey: Blackwell; 2002. p. 650.
11. Schallhorn RG, Hiatt WH, Boyce W. Iliac transplants in periodontal therapy. J Periodontol ١٩٧٠Oct;41(10):566-8
12. Dragoo MR, Sullivan HC. A clinical and histological evaluation of autogenous iliac bone grafts in humans. I. Wound healing 2 to 8 months. J Periodontol 1973 Oct;44(10):599-613.
13. Schallhorn RG. Postoperative problems associated with iliac transplants. J Periodontol ١٩٧٢Jan;43(1):3-9
14. Froum SJ, Thaler R, Scopp IW, Stahl SS Osseous autografts. I. Clinical responses to bone blend or hip marrow grafts. J Periodontol 1975 Sep;46(9):515-21

15. Moskow BS, Karsh F, Stein SD. Histological assessment of autogenous bone graft. A case report

and critical evaluation. J Periodontol 1979 Jun؛

.٣٠٠-٢٩١:(٦)٥٠

16. Listgarten MA, Rosenberg MM. Histological

study of repair following new attachment procedures in human periodontal lesions. J Periodontol

١٩٧٩Jul;50(7):333-44

17. Quattlebaum JB, Mellonig JT, Hensel NF.

Antigenicity of freeze-dried cortical bone allograft in human periodontal osseous defects. J Periodontol 1988 Jun;59(6):394-7.

18. Mabry TW, Yukna RA, Sepe WW. Freezedried bone allografts combined with tetracycline in the treatment of juvenile periodontitis. J Periodontol 1985 Feb;56(2):74-81

19. Evans GH, Yukna RA, Sepe WW, Mabry TW، Mayer ET. Effect of various graft materials with tetracycline in localized juvenile periodontitis. J Periodontol 1989 Sep;60(9):491-7

20. Mellonig JT, Bowers GM, Bailey RC. Comparison of bone graft materials. Part I. Newbone formation with autografts and

allografts determined by Strontium-85. J Periodontol 1981 Jun;52(6):291-6.

21. Urist MR, Strates BS. Bone morphogenetic protein. J Dent Res 1971;50(6):1392-406 -٢٦ .Tsao YP, Neiva R, Al-Shammari K, Oh TJ،

Wang HL. Effects of a mineralized human cancellous bone allograft in regeneration of mandibular Class II furcation defects. J Periodontol 2006

22. Spector M. Anorganic bovine bone and ceramic analogs of bone mineral as implants tofacilitate bone regeneration. Clin Plast Surg 1994

Jul;21(3):437-44

23. Mellonig JT. Human histologic evaluation of a bovine-derived bone xenograft in the treatment of periodontal osseous defects. Int J Periodontics Restorative Dent 2000 Feb;20(1):19-29.

24. Nevins ML, Camelo M, Lynch SE, Schenk

RK, Nevins M. Evaluation of periodontal regeneration following grafting intrabony defects withbio-oss collagen: a human histologic report. Int J

Periodontics Restorative Dent 2003;23(1):9-17

25. Maiorana C, Santoro F, Rabagliati M, Salina S. Evaluation of the use of iliac cancellous bone and anorganic bovine bone in the reconstruction of the atrophic maxilla with titanium mesh: a clinical and histologic investigation. Int J Oral Maxillofac Implants 2001 May-Jun;16(3):427-32

26. Valentini P, Abensur D, Wenz B, Peetz M Schenk R. Sinus grafting with porous bone mineral)Bio-Oss) for implant placement: a 5-year study on ١٥patients. Int J Periodontics Restorative Dent 2000 Jun;20(3):245-53.

27. Orsini G, Scarano A, Piattelli M, Piccirilli M Caputi S, Piattelli A. Histologic and ultrastructuralanalysis of regenerated bone in maxillary sinusaugmentation using a porcine bone-derived biomaterial. J Periodontol 2006 Dec;77(12):1984-90

28. Yukna RA, Mayer ET, Amos SM. 5-year evaluation of durapatite ceramic alloplastic implants in periodontal osseous defects. J Periodontol 1989 Oct;60(10):544-51

29. Lekovic V, Kenney EB, Carranza FA Jr، Danilovic V. Treatment of class II furcation defects using porous hydroxylapatite in conjunction with a polytetrafluoroethylene membrane. J Periodontol 1990 Sep;61(9):575-8

30. Yukna RA. HTR polymer grafts in human periodontal osseous defects. I. 6-month clinical results. J Periodontol 1990 Oct;61(10):633-42

31. Baldock WT, Hutchens LH Jr, McFall WT Jr Simpson DM. An evaluation of tricalcium phosphate implants in human periodontal osseous defects of two patients. J Periodontol 1985 Jan;56.٧-١:(١)

32. Stahl SS, Froum S. Histological evaluation of human intraosseous healing responses to the placement of tricalcium phosphate ceramic implants. I. Three to eight months. J Periodontol 1986 Apr;57(4):211-7

33. Kenney EB, Lekovic V, Sa Ferreira JC, Han T,Dimitrijevic B, Carranza FA Jr. Bone formation within porous hydroxylapatite implants in human periodontal defects. J Periodontol 1986 Feb;57 .٨٣-٧٦:(٢)

34. Stahl SS, Froum SJ, Tarnow D. Human clinical and histologic responses to the placement of HTR polymer particles in 11 intrabony lesions. J Periodontol 1990 May;61(5):269-74

35. Poehling S, Pippig SD, Hellerbrand K, Siedler M, Schütz A, Dony C. Superior effect of MD05‹ beta-tricalcium phosphate coated with recombinant human growth/differentiation factor-5,

compared to conventional bone substitutes in the rat calvarial defect model. J Periodontol 2006;77(9):1582-90

36. Froum SJ, Weinberg MA, Tarnow D. Comparison of bioactive glass synthetic bone graft particles and open debridement in the treatment of

human periodontal defects. A clinical study. J Periodontol 1998 Jun;69(6):698-709 37. Nishida T, Yamada Y, Murai M, Shimizu Y

Oshikawa M, Ito K. Effects of bioactive glass on bone augmentation within a titanium cap in rabbit parietal bone. J Periodontol 2006 Jun;77(6):983-9

38. Mengel R, Schreiber D, Flores-de-Jacoby LBioabsorbable membrane and bioactive glass in the treatment of intrabony defects in patients with

generalized aggressive periodontitis: results of a 5- year clinical and radiological study. J Periodontol ٢٠٠٦Oct;77(10):1781-7.

39. AH. Melcher, J. Periodontol., 1976, 47, 256-260.

40. T. Karring, P. Cortellini, Periodontol. 2000, 1999, 19, 115-137.

41. S. Nyman, J. Lindhe, T. Karring, H. Rylander, J. Clin. Periodontol., 1982, 9, 290-296.

42. TV. Scantlebury, J. Periodontol., 1993, 64, 1129-1137.

43. J. Black; Fundamentals of Biocompatibility. Biological Performance of Materials, New York, 1992, 3-9

44. DF. Williams, In: DF. Williams (Ed.), Fundamental Aspects of Biocompatibility (CRC Press, Boca Raton, 1981) 2-7

45. FC. Usher, SA. Wallace, Arch. Surg., 1958, 76, 997-999

46. J. Gottlow, S. Nyman, J. Lindhe, T. Karring, J. Wennstrom, J. Clin. Periodontol., 1986, 13, 604- 616

47. P. Cortellini, G. Pini Prato, MS. Tonetti , J. Periodontol., 1993, 64, 261-268

48. KG. Murphy, Int. J. Periodontics Rest. Dent., 1995, 15, 363-375

49. I. Aukhil, E. Pettersson, C. Suggs, J. Periodontol ., 1986, 57, 727-734

50. M. Minabe, J. Periodontol., 1991, 62, 171-179

51.Karring T, Nyman S, Lindhe J. Healing following implantation of periodontitis affected roots into bone tissue. J Clin Period- ontol 1980;7:96–105 .

52. Nyman S, Karring T, Lindhe J, Planten S. Healing following implantation of periodontitis-affected roots into gingival connective tissue. J Clin Periodontol 1980;7:394–401.

53. Gottlow J, Nyman S, Lindhe J, Karring T, Wennstrom J. New attachment formation in the human periodontium by guided tissue regeneration. Case reports. J Clin Periodontol 1986;13: 60 .

54. Prichard J. Regeneration of bone following periodontal therapy:report of cases. Oral Surg Oral Med Oral Pathol 1957;10:247– 252 .

55. Caton J, Nyman S, Zander H. Histometric evaluation of peri-odontal surgery. II. Connective tissue attachment levels after four regenerative procedures. J Clin Periodontol 1980;7:224–231 .

56. Reynolds MA ,

Aichelmann-Reidy ME, Branch-Mays GL, Gun- solley JC. The efficacy of replacement grafts in the treatment of periodontal osseous defects.A systematic review. Ann Periodontol 2003;8:227–265.

57. Tonetti MS, Pini Prato G Stalpers G, Cortellini P. Guided tissue regeneration of deep intrabony defects in strategically important prosthetic abutments. Int J Periodontics Restorative Dent 1996;16:378–387.

58. Chung KM, Salkin LM, Stein MD, Freedman AL. Clinical evaluation of a biodegradable collagen membrane in guided

tissue regeneration. J Periodontol 1990;61:732–736.

.

59. Tonetti MS, Pini-Prato G,Cortellini P. Effect of cigarette smoking on periodontal healing following GTR in infrabony defects. A preliminary retrospective study. J Clin Periodontal 1995;22:229–234. .

60. Tonetti MS, Prato GP, Cortellini P. Factors affecting the healing response of intrabony defects following guided tissue regeneration and access flap surgery. J Clin Periodontol 1996;23:548–556.

61. Ehnevid H, Jansson L, Lindskog S, Blomlof L. Periodontal healing in teeth with periapical lesions. A clinical retrospective study. J Clin Periodontol 1993;20:254–258.

62. Ehnevid H, Jansson LE, Lindskog SF, Blomlof LB. Periodontal healing in relation to radiographic attachment and endodontic infection. J Periodontol 1993;64:1199–1204.

63. Cortellini P, Tonetti MS. Evaluation of the effect of tooth vitality on regenerative outcomes in infrabony defects. J Clin Period-ontol 2001;28:672–679.

64. Cortellini P, Tonetti MS, Lang NP, et al. The simplified papillae preservation flap in the regenerative treatment of deep

intrabony defects: clinical outcomes and postoperative morbidity.J Periodontol 2001;72:1702–1712.

65. Trejo PM, Weltman RL. Favorable periodontal regenerative outcomes from teeth with presurgical mobility: a retrospective study. J Periodontol 2004;75:1532–1538.

66. Tonetti MS, Pini-Prato G, Cortellini P. Periodontal regeneration of human intrabony defects. IV. Determinants of healing response. J Periodontol 1993;64:934–940.

67. Linares A, Cortellini P, Lang NP, Suvan J, Tonetti MS. Guided tissue regeneration/deproteinized bovine bone mineral or papilla preservation flaps alone for treatment of intrabony defects. II: radiographic predictors and outcomes. J Clin Periodontol 2006;33:351–358.

68. Murphy KG. Postoperative healing complications associated with Gore-Tex Periodontal Material. Part I. Incidence and characterization. Int J Periodontics Restorative Dent 1995;15:363–375.

69. Selvig KA, Kersten BG, Chamberlain AD, Wikesjo UM, Nilveus RE. Regenerative surgery of intrabony periodontal defects using ePTFE barrier membranes: scanning electron

microscopic evaluation of retrieved membranes versus clinical healing. J Period- ontol 1992;63:974–978

.

70. Cortellini P, Prato GP, Tonetti MS. The modified papilla preservation technique. A new surgical approach for interproximal regenerative procedures. J Periodontol 1995;66:261–266. .

71. Cortellini P, Prato GP, Tonetti MS. The simplified papilla pres-ervation flap. A novel surgical approach for the management of soft tissues in regenerative procedures. Int J Periodontics Restorative Dent 1999;19:589–599.

72. Melcher , (1986)

73.Howard et al , (1998)

74.schroeder , (1986)

75.Abiko and shimono, 1989,Chen et al , 1989 ;inoue et al ; 1981 ,1986 a,b 1990 , 1992 , 1993 a; shimono , 1991; shimono et al , 1988 ; Yamamura et al , 1980

76.caton et al , 1992; caton and Greenstein , 1993 ; caton , 1997 ; Gould et al . 1977 , 1980 ; karring and warrer , 1992 ; Lindhe et al .,1995 ; Nymann et al , 1982 , 1983 , sander and karring , 1995 a, b ;sodek , 1977

77.Cochran and *wozney*, 1999

78.worapamorn et al , 2000

79.shimazu et al , 1999

80.Hammarstrom , 1997a , 1997b ; Hammar-strom et al ; 1996

81.Andreasen , et al ; 1995 a,b,c krasner and Rankow , 1995 ; kratchman , 1997 ; Nguyen et al , 1992 , 1997 ; Tsukiboshi , 1993

82.Iqbal and bamas , 2001, kawanami , et al , 2001 ; Lekic et al ; 2001 ; lin et al 2000

83.Beertsen et al , 1997 , Inoue et al 1995a

84.Boyko GA , Melcher AH , Brunette DM . Formation of new periodontal ligament by periodontal ligament cells implanted in vivid after culture in vitro . A preliminary study of transplanted roots in the dog . J periodontal Res 1981 ; 16 : 73-88

85.Karring T, Nyman S, Lindhe J. Healing following implantation of periodontitis affected roots into bone tissue. J Clin Periodontol 1980; 7: 96–105

86.newman and carranza's clinical periodontology

87.Richard T. Kao, Henry H. Takei, David L. Cochran and Marc L. Nevins

91- Pihlstrom, B.L.; Michalowicz, B.S.; Johnson, N.W. Periodontal diseases. Lancet 2005, 366, 1809–1820. [Google Scholar] [CrossRef] [PubMed]

92- Matichescu, A.; Ardelean, L.C.; Rusu, L.C.; Craciun, D.; Bratu, E.A.; Babucea, M.; Leretter, M. Advanced Biomaterials and Techniques for Oral Tissue Engineering and Regeneration—A Review. Materials 2020, 13, 5303. [Google Scholar] [CrossRef] [PubMed]

93- Bernardi, S.; Macchiarelli, G.; Bianchi, S. Autologous Materials in Regenerative Dentistry: Harvested Bone, Platelet Concentrates and Dentin Derivates. Molecules 2020, 25, 5330

. [Google Scholar] [CrossRef] [PubMed]

94- Hollý, D.; Klein, M.; Mazreku, M.; Zamborský, R.; Polák, Š.; Danišovič, Ľ.; Csöbönyeiová, M. Stem Cells and Their Derivatives-Implications for Alveolar Bone Regeneration: A Comprehensive Review. Int. J. Mol. Sci. 2021, 22, 11746. [Google Scholar] [CrossRef] [PubMed]

95- Jin, S.; Xia, X.; Huang, J.; Yuan, C.; Zuo, Y.; Li, Y.; Li, J. Recent advances in PLGA-based biomaterials for bone tissue

regeneration. Acta Biomater. 2021, 127, 56–79. [Google Scholar] [CrossRef]

96- Vouvoudi, E.C. Overviews on the Progress of Flowable Dental Polymeric Composites: Their Composition, Polymerization Process, Flowability and Radiopacity Aspects. Polymers 2022, 14, 4182. [Google Scholar] [CrossRef]

97- Valandro, L.F.; Cadore-Rodrigues, A.C.; Dapieve, K.S.; Machry, R.V.; Pereira, G.K.R. A brief review on fatigue test of ceramic and some related matters in Dentistry. J. Mech. Behav. Biomed. Mater. 2023, 138, 105607. [Google Scholar] [CrossRef]

98- Albeshir, E.G.; Alsahafi, R.; Albluwi, R.; Balhaddad, A.A.; Mitwalli, H.; Oates, T.W.; Hack, G.D.; Sun, J.; Weir, M.D.; Xu, H.H.K. Low-Shrinkage Resin Matrices in Restorative Dentistry-Narrative Review. Materials 2022, 15, 2951. [Google Scholar] [CrossRef]

99- Abid, N.; Khan, A.M.; Shujait, S.; Chaudhary, K.; Ikram, M.; Imran, M.; Haider, J.; Khan, M.; Khan, Q.; Maqbool, M. Synthesis of nanomaterials using various top-down and bottom-up approaches, influencing factors, advantages, and

disadvantages: A review. Adv. Colloid Interface Sci. 2022, 300, 102597. [Google Scholar] [CrossRef]

100- Qiu, J.; Liu, X.-J.; You, B.-A.; Ren, N.; Liu, H. Application of Nanomaterials in Stem Cell-Based Therapeutics for Cardiac Repair and Regeneration. Small 2023, 19, 2206487. [Google Scholar] [CrossRef]

101- Hu, T.; Gu, Z.; Williams, G.R.; Strimaite, M.; Zha, J.; Zhou, Z.; Zhang, X.; Tan, C.; Liang, R. Layered double hydroxide-based nanomaterials for biomedical applications. Chem. Soc. Rev. 2022, 51, 6126–6176. [Google Scholar] [CrossRef] [PubMed]

102- Armenia, I.; Cuestas Ayllón, C.; Torres Herrero, B.; Bussolari, F.; Alfranca, G.; Grazú, V.; Martínez de la Fuente, J. Photonic and magnetic materials for on-demand local drug delivery. Adv. Drug Deliv. Rev. 2022, 191, 114584. [Google Scholar] [CrossRef] [PubMed]

103- Zong, C.; Van Holm, W.; Bronckaers, A.; Zhao, Z.; Čokić, S.; Aktan, M.K.; Castro, A.B.; Van Meerbeek, B.; Braem, A.; Willems, G.; et al. Biomimetic Periodontal Ligament Transplantation Activated by Gold Nanoparticles Protects

Alveolar Bone. Adv. Healthc. Mater. 2023, 2300328. [Google Scholar] [CrossRef] [PubMed]

104- Dadfar, S.M.; Roemhild, K.; Drude, N.I.; von Stillfried, S.; Knüchel, R.; Kiessling, F.; Lammers, T. Iron oxide nanoparticles: Diagnostic, therapeutic and theranostic applications. Adv. Drug Deliv. Rev. 2019, 138, 302–325. [Google Scholar] [CrossRef] [PubMed]

105- Ding, Q.; Cui, J.; Shen, H.; He, C.; Wang, X.; Shen, S.G.F.; Lin, K. Advances of nanomaterial applications in oral and maxillofacial tissue regeneration and disease treatment. Wiley Interdiscip. Rev. Nanomed. Nanobiotechnol. 2020, 13, e1669. [Google Scholar] [CrossRef]

106- Feng, W.; Chen, Y. Chemoreactive nanomedicine. J. Mater. Chem. B 2020, 8, 6753–6764. [Google Scholar] [CrossRef]

107- Lenders, V.; Koutsoumpou, X.; Sargsian, A.; Manshian, B.B. Biomedical nanomaterials for immunological applications: Ongoing research and clinical trials. Nanoscale Adv. 2020, 2, 5046–5089. [Google Scholar] [CrossRef]

108- Tiwari, N.; Osorio-Blanco, E.R.; Sonzogni, A.; Esporrín-Ubieto, D.; Wang, H.; Calderón, M. Nanocarriers for Skin Applications: Where Do We Stand? Angew. Chem. Int. Ed. Engl. 2022, 61, e202107960. [Google Scholar] [CrossRef]

109- cancer therapy: Current progress and perspectives. J. Hematol. Oncol. 2021, 14, 85. [Google Scholar] [CrossRef]

110- Lewis, J.S.; Eniola-Adefeso, O. Polymeric particle-based therapies for acute inflammatory diseases. Nat. Rev. Mater. 2022, 7, 796–813. [Google Scholar] [CrossRef]

111- Su, S.; Kang, P.M.

Systemic Review of Biodegradable Nanomaterials in Nanomedicine. Nanomaterials 2020, 10, 656. [Google Scholar] [CrossRef] [PubMed]

112- Avila-Ortiz, G.; Elangovan, S.; Kramer, K.W.; Blanchette, D.; Dawson, D.V. Effect of alveolar ridge preservation after tooth extraction: A systematic review and meta-analysis. J. Dent. Res. 2014, 93, 950–958. [Google Scholar] [CrossRef] [PubMed]

113- Sheikh, Z.; Hamdan, N.; Ikeda, Y.; Grynpas, M.; Ganss, B.; Glogauer, M. Natural graft tissues and synthetic biomaterials for periodontal and alveolar bone reconstructive applications: A review. Biomater. Res. 2017, 21, 9. [Google Scholar] [CrossRef] [PubMed]

114- Ren, Y.; Fan, L.; Alkildani, S.; Liu, L.; Emmert, S.; Najman, S.; Rimashevskiy, D.; Schnettler, R.; Jung, O.; Xiong, X.; et al. Barrier Membranes for Guided Bone Regeneration (GBR): A Focus on Recent Advances in Collagen Membranes. Int. J. Mol. Sci. 2022, 23, 14987. [Google Scholar] [CrossRef]

115- Sun, J.; Hu, Y.; Fu, Y.; Zou, D.; Lu, J.; Lyu, C. Emerging roles of platelet concentrates and platelet-derived extracellular vesicles in regenerative periodontology and implant dentistry. APL Bioeng. 2022, 6, 031503. [Google Scholar] [CrossRef]

116- Arzate, H.; Zeichner-David, M.; Mercado-Celis, G. Cementum proteins: Role in cementogenesis, biomineralization, periodontium formation and regeneration. Periodontol. 2000 2015, 67, 211–233. [Google Scholar] [CrossRef]

117- Miron, R.J.; Moraschini, V.; Fujioka-Kobayashi, M.; Zhang, Y.; Kawase, T.; Cosgarea, R.; Jepsen, S.; Bishara, M.; Canullo, L.; Shirakata, Y.; et al. Use of platelet-rich fibrin for

the treatment of periodontal intrabony defects: A systematic review and meta-analysis. Clin. Oral Investig. 2021, 25, 2461–2478. [Google Scholar] [CrossRef]

118- Zakrzewski, W.; Dobrzynski, M.; Rybak, Z.; Szymonowicz, M.; Wiglusz, R.J. Selected Nanomaterials' Application Enhanced with the Use of Stem Cells in Acceleration of Alveolar Bone Regeneration during Augmentation Process. Nanomaterials 2020, 10, 1216. [Google Scholar] [CrossRef]

119- Funda, G.; Taschieri, S.; Bruno, G.A.; Grecchi, E.; Paolo, S.; Girolamo, D.; Del Fabbro, M. Nanotechnology Scaffolds for Alveolar Bone Regeneration. Materials 2020, 13, 201. [Google Scholar] [CrossRef]

120- Iviglia, G.; Kargozar, S.; Baino, F. Biomaterials, Current Strategies, and Novel Nano-Technological Approaches for Periodontal Regeneration. J. Funct. Biomater. 2019, 10, 3. [Google Scholar] [CrossRef]

121- Al-Ahmady, H.H.; Abd Elazeem, A.F.; Bellah Ahmed, N.E.; Shawkat, W.M.; Elmasry, M.; Abdelrahman, M.A.; Abderazik, M.A. Combining autologous bone marrow mononuclear cells seeded on collagen sponge with Nano

Hydroxyapatite, and platelet-rich fibrin: Reporting a novel strategy for alveolar cleft bone regeneration. J. Craniomaxillofac. Surg. 2018, 46, 1593–1600. [Google Scholar] [CrossRef] [PubMed]

122- Nair, M.B. Nanofibrous yarn reinforced HA-gelatin composite scaffolds promote bone formation in critical sized alveolar defects in rabbit model. Biomed. Mater. 2018, 13, 065011. [Google Scholar] [CrossRef] [PubMed]

123- Fang, C.H.; Sun, C.K.; Lin, Y.W.; Hung, M.C.; Lin, H.Y.; Li, C.H.; Lin, I.P.; Chang, H.C.; Sun, J.S.; Chang, J.Z. Metformin-Incorporated Gelatin/Nano-Hydroxyapatite Scaffolds Promotes Bone Regeneration in Critical Size Rat Alveolar Bone Defect Model. Int. J. Mol. Sci. 2022, 23, 558. [Google Scholar] [CrossRef] [PubMed]

124- Fang, C.H.; Lin, Y.W.; Lin, F.H.; Sun, J.S.; Chao, Y.H.; Lin, H.Y.; Chang, Z.C. Biomimetic Synthesis of Nanocrystalline Hydroxyapatite Composites: Therapeutic Potential and Effects on Bone Regeneration. Int. J. Mol. Sci. 2019, 20, 6002. [Google Scholar] [CrossRef] [PubMed]

125- Han, J.; Ma, B.; Liu, H.; Wang, T.; Wang, F.; Xie, C.; Li, M.; Liu, H.; Ge, S. Hydroxyapatite nanowires modified

polylactic acid membrane plays barrier/osteoinduction dual roles and promotes bone regeneration in a rat mandible defect model. J. Biomed. Mater. Res. A 2018, 106, 3099–3110. [Google Scholar] [CrossRef]

126- Calasans-Maia, M.D.; Barboza, C.A.B., Jr.; Soriano-Souza, C.A.
; Alves, A.; Uzeda, M.J.P.; Martinez-Zelaya, V.R.; Mavropoulos, E.; Rocha Leão, M.H.; de Santana, R.B.; Granjeiro, J.M.; et al. Microspheres of alginate encapsulated minocycline-loaded nanocrystalline carbonated hydroxyapatite: Therapeutic potential and effects on bone regeneration. Int. J. Nanomed. 2019, 14, 4559–4571. [Google Scholar] [CrossRef]

127- Dhawan, S.; Takiar, M.; Manocha, A.; Dhawan, R.; Malhotra, R.; Gupta, J. Functionally graded membrane: A novel approach in the treatment of gingival recession defects. J. Indian Soc. Periodontol. 2021, 25, 411–417. [Google Scholar] [CrossRef]

128- Mansour, A.M.; Yahia, S.; Elsayed, H.R.H.; El-Attar, S.A.E.; Grawish, M.E.; El-Hawary, Y.M.; El-Sherbiny, I.M. Efficacy of biocompatible trilayers nanofibrous scaffold with/without allogeneic adipose-derived stem cells on class II

furcation defects of dogs' model. Clin. Oral Investig. 2022, 26, 2537–2553. [Google Scholar] [CrossRef]

129- Yan, N.; Hu, B.; Xu, J.; Cai, R.; Liu, Z.; Fu, D.; Huo, B.; Liu, Z.; Zhao, Y.; Chen, C.; et al. Stem cell Janus patch for periodontal regeneration. Nano Today 2022, 42, 101336. [Google Scholar] [CrossRef]

130- Zhou, T.; Liu, X.; Sui, B.; Liu, C.; Mo, X.; Sun, J. Development of fish collagen/bioactive glass/chitosan composite nanofibers as a GTR/GBR membrane for inducing periodontal tissue regeneration. Biomed. Mater. 2017, 12, 055004. [Google Scholar] [CrossRef]

131- Boda, S.K.; Almoshari, Y.; Wang, H.; Wang, X.; Reinhardt, R.A.; Duan, B.; Wang, D.; Xie, J. Mineralized nanofiber segments coupled with calcium-binding BMP-2 peptides for alveolar bone regeneration. Acta Biomater. 2019, 85, 282–293. [Google Scholar] [CrossRef] [PubMed]

132- Boda, S.K.; Wang, H.; John, J.V.; Reinhardt, R.A.; Xie, J. Dual Delivery of Alendronate and E7-BMP-2 Peptide via Calcium Chelation to Mineralized Nanofiber Fragments for Alveolar Bone Regeneration. ACS Biomater. Sci. Eng. 2020, 6, 2368–2375. [Google Scholar] [CrossRef] [PubMed]

133- Yu, M.; Luo, D.; Qiao, J.; Guo, J.; He, D.; Jin, S.; Tang, L.; Wang, Y.; Shi, X.; Mao, J.; et al. A hierarchical bilayer architecture for complex tissue regeneration. Bioact. Mater. 2022, 10, 93–106. [Google Scholar] [CrossRef]

134- Shaikh, M.S.; Zafar, M.S.; Alnazzawi, A. Comparing Nanohydroxyapatite Graft and Other Bone Grafts in the Repair of Periodontal Infrabony Lesions: A Systematic Review and Meta-Analysis. Int. J. Mol. Sci. 2021, 22, 12021. [Google Scholar] [CrossRef] [PubMed]

135- Wu, D.T.; Munguia-Lopez, J.G.; Cho, Y.W.; Ma, X.; Song, V.; Zhu, Z.; Tran, S.D. Polymeric Scaffolds for Dental, Oral, and Craniofacial Regenerative Medicine. Molecules 2021, 26, 7043. [Google Scholar] [CrossRef]

136- Xing, D.; Zuo, W.; Chen, J.; Ma, B.; Cheng, X.; Zhou, X.; Qian, Y. Spatial Delivery of Triple Functional Nanoparticles via an Extracellular Matrix-Mimicking Coaxial Scaffold Synergistically Enhancing Bone Regeneration. ACS Appl. Mater. Interfaces 2022, 14, 37380–37395. [Google Scholar] [CrossRef]

137- Sowmya, S.; Mony, U.; Jayachandran, P.; Reshma, S.; Kumar, R.A.; Arzate, H.; Nair, S.V.; Jayakumar, R. Tri-Layered Nanocomposite Hydrogel Scaffold for the Concurrent Regeneration of Cementum, Periodontal Ligament, and Alveolar Bone. Adv. Healthc. Mater. 2017, 6, 1601251. [Google Scholar] [CrossRef]

138- Shang, L.; Liu, Z.; Ma, B.; Shao, J.; Wang, B.; Ma, C.; Ge, S. Dimethyloxallyl glycine/nanosilicates-loaded osteogenic/angiogenic difunctional fibrous structure for functional periodontal tissue regeneration. Bioact. Mater. 2021, 6, 1175–1188. [Google Scholar] [CrossRef]

139- Xue, Y.; Hong, X.; Gao, J.; Shen, R.; Ye, Z. Preparation and biological characterization of the mixture of poly(lactic-co-glycolic acid)/chitosan/Ag nanoparticles for periodontal tissue engineering. Int. J. Nanomed. 2019, 14, 483–498. [Google Scholar] [CrossRef]

140- Stutz, C.; Strub, M.; Clauss, F.; Huck, O.; Schulz, G.; Gegout, H.; Benkirane-Jessel, N.; Bornert, F.; Kuchler-Bopp, S. A New Polycaprolactone-Based Biomembrane Functionalized with BMP-2 and Stem Cells Improves Maxillary Bone Regeneration.

Nanomaterials 2020, 10, 1774. [Google Scholar] [CrossRef]

141- Sun, M.; Liu, Y.; Jiao, K.; Jia, W.; Jiang, K.; Cheng, Z.; Liu, G.; Luo, Y. A periodontal tissue regeneration strategy via biphasic release of zeolitic imidazolate framework-8 and FK506 using a uniaxial electrospun Janus nanofiber. J. Mater. Chem. B 2022, 10, 765–778. [Google Scholar] [CrossRef] [PubMed]

142- Jiang, Y.; Liu, J.M.; Huang, J.P.; Lu, K.X.; Sun, W.L.; Tan, J.Y.; Li, B.X.; Chen, L.L.; Wu, Y.M. Regeneration potential of decellularized periodontal ligament cell sheets combined with 15-deoxy-Δ(12,14)-prostaglandin J(2) nanoparticles in a rat periodontal defect. Biomed. Mater. 2021, 16, 045008. [Google Scholar] [CrossRef] [PubMed]

143- Batool, F.; Strub, M.; Petit, C.; Bugueno, I.M.; Bornert, F.; Clauss, F.; Huck, O.; Kuchler-Bopp, S.; Benkirane-Jessel, N. Periodontal Tissues, Maxillary Jaw Bone, and Tooth Regeneration Approaches: From Animal Models Analyses to Clinical Applications. Nanomaterials 2018, 8, 337. [Google Scholar] [CrossRef] [PubMed]

144- Martins, C.; Sousa, F.; Araújo, F.; Sarmento, B. Functionalizing PLGA and PLGA Derivatives for Drug

Delivery and Tissue Regeneration Applications. Adv. Healthc. Mater. 2018, 7, 1701035. [Google Scholar] [CrossRef]

145- Bharadwaz, A.; Jayasuriya, A.C. Recent trends in the application of widely used natural and synthetic polymer nanocomposites in bone tissue regeneration. Mater. Sci. Eng. C. Mater. Biol. Appl. 2020, 110, 110698. [Google Scholar] [CrossRef]

146- Cheheltani, R.; Ezzibdeh, R.M.; Chhour, P.; Pulaparthi, K.; Kim, J.; Jurcova, M.; Hsu, J.C.; Blundell, C.; Litt, H.I.; Ferrari, V.A.; et al. Tunable, biodegradable gold nanoparticles as contrast agents for computed tomography and photoacoustic imaging. Biomaterials 2016, 102, 87–97. [Google Scholar] [CrossRef]

147- Zong, C.; Bronckaers, A.; Vande Velde, G.; Willems, G.; Cadenas de Llano-Pérula, M. In Vivo Micro-Computerized Tomography Tracking of Human Periodontal Ligament Stem Cells Labeled with Gold Nanocomplexes. Adv. Healthc. Mater. 2022, 11, e2101133. [Google Scholar] [CrossRef]

148- Tharmalingam, N.; Bose, R.J.; Park, H.; Ha, D.H. Labeling and tracking cells with gold nanoparticles. Drug Discov. Today 2021, 26, 94–105. [Google Scholar] [CrossRef]

149- Li, L.; Zhang, Y.; Wang, M.; Zhou, J.; Zhang, Q.; Yang, W.; Li, Y.; Yan, F. Gold Nanoparticles Combined Human β-Defensin 3 Gene-Modified Human Periodontal Ligament Cells Alleviate Periodontal Destruction via the p38 MAPK Pathway. Front. Bioeng. Biotechnol. 2021, 9, 631191. [Google Scholar] [CrossRef]

150- Ni, C.; Zhou, J.; Kong, N.; Bian, T.; Zhang, Y.; Huang, X.; Xiao, Y.; Yang, W.; Yan, F. Gold nanoparticles modulate the crosstalk between macrophages and periodontal ligament cells for periodontitis treatment. Biomaterials 2019, 206, 115–132. [Google Scholar] [CrossRef]

151- Zhang, Y.; Kong, N.; Zhang, Y.; Yang, W.; Yan, F. Size-dependent Effects of Gold Nanoparticles on Osteogenic Differentiation of Human Periodontal Ligament Progenitor Cells. Theranostics 2017, 7, 1214–1224. [Google Scholar] [CrossRef] [PubMed]

152- Yi, C.; Liu, D.; Fong, C.-C.; Zhang, J.; Yang, M. Gold nanoparticles promote osteogenic differentiation of mesenchymal stem cells through p38 MAPK pathway. ACS Nano 2010, 4, 6439–6448. [Google Scholar] [CrossRef]