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Bioactive Scaffolds for Alveolar Bone Regeneration

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ABSTRACT

Periodontitis is an inflammatory condition leading to progressive destruction of periodontal tissues, and is a major cause of tooth loss in adults. Alveolar bone resorption jeopardizes the structural and functional integrity of the periodontium and also affects the esthetic outcomes of implant treatment. New attachment with periodontal regeneration is considered to be the ideal outcome of periodontal therapy resulting in reconstruction of the periodontium. Guided bone regeneration (GBR) is assumed to be achieved when the osteoprogenitor cells are exclusively allowed to repopulate the bone defect site by preventing the entry of non-osteogenic tissues. Although the conventional membranes act as a physical barrier for preventing apical migration of epithelial and gingival connective tissue cells, they possess many bio-functional limitations. So, in order to overcome these drawbacks and to enhance the bioactivity it is necessary to incorporate other bioactive materials and nanoparticles. In this review we have attempted to highlight the new trends in membrane modification by the use of bioactive nanoparticles and bioactive molecules.

Keywords: Periodontitis, Osseous defects, Guided tissue regeneration, Guided bone regeneration, Bioactive materials, Nanoparticles

Abbreviations: GTR: Guided Tissue Regeneration; GBR: Guided Bone Regeneration; E-PTFE: Expanded Polytetrafluoroethylene; PGA: Polyglycolic Acid; PLA: Polylactic Acid; PTFE: D-PTFE: High Density; SEM: Scanning Electron Microscopy; FTIR-ATR: Attenuated Total Reflectance- Fourier Transform Infrared Spectroscopy; XRD: X-Ray Diffraction; DBBM: Deproteinized Bovine Bone Mineral; HA: Hydroxyapatite; B-TCP: B-Tricalcium Phosphate; BCF: Biphasic Calcium Phosphate; EMD: Emdogain; BMPS: Bone Morphogenic Proteins; FGF-2: Fibroblast Growth Factor; PDGF: Platelet-Derived Growth Factor; IGF: Insulin-Like Growth Factor; BMP: Bone Morphogenetic Protein; TGF: Transforming Growth Factor; OP: Osteogenic Protein; BDNF: Brain-Derived Neurotrophic Factor

INTRODUCTION

The periodontium is a functional unit which is composed of gingiva, periodontal ligament, cementum and alveolar bone. Periodontitis is an inflammatory condition that leads to progressive destruction of periodontal tissues and is a major cause of tooth loss in adults [1]. Periodontal therapy includes scaling, root planning, curettage and flap procedures and these are characterized by healing mainly by repair with little or no regeneration. New attachment with periodontal regeneration is the ideal outcome of periodontal therapy and results in reconstruction of the periodontium [2].

By definition, periodontal regeneration is the formation of new cementum, periodontal ligament, and alveolar bone so that the form and function of the lost structures are restored [3]. Regenerative procedures include root biomodification, guided tissue regeneration and bone grafts. Following flap surgery, the curetted root surface maybe repopulated by epithelial cells, gingival connective tissue cells, bone cells and periodontal ligament cells. The concept of placing barriers of different types is to prevent migration of epithelial cells into the wound and to favor repopulation of the area by cells from the periodontal ligament and bone cells [2]. Alveolar bone resorption jeopardizes the structural and functional integrity of the periodontium and the esthetic outcomes of implant treatment [3]. For achieving good long-term prognosis for Osseo integrated implants, a

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sufficient volume of bone must exist at the site of implantation [4-7]. Osseous defects can be managed with simultaneous implant placement in implant dehiscence or fenestration defect in which defects are corrected using barrier membranes [8]. Controlled study in humans has shown better results in the membrane treated groups and resulted in 95 to 100 % elimination of dehiscence defects [9]. Guided bone regeneration (GBR) is one of the most common method used for reconstruction of alveolar bone and for treating peri implant bone deficiencies [10] and involves membrane placement in a bony defect to exclude non - osteogenic tissues from interfering with bone regeneration. Around 40% of Osseo integrated implants require GBR as a part of patient's rehabilitation [11]. The American Academy of Periodontology has defined guided tissue regeneration (GTR) as "the procedure by which a barrier is utilized to exclude epithelium from the root surfaces". This method is derived from the classic studies of Nyman (1984)[12], Lindhe (1984), Karring (1986) and is based on the assumption that only the periodontal ligament cells have the potential for regeneration of the attachment apparatus of the tooth [2].

Minabe [13] classified GTR membranes as bioabsorbable and non-absorbable. Bioabsorbable membranes are again classified as natural and synthetic. Natural bioabsorbable membranes include collagen membrane, connective tissue graft, oxidized cellulose graft, synthetic polymer and duramater. Synthetic bioabsorbable membranes include alloderm, polylactic acid membrane, polyglycolic acid membrane. No absorbable membranes include expanded polytetrafluoroethylene, rubber dam, titanium reinforced polytetrafluoroethylene. Selecting ideal biomaterial for GTR membrane include following requirements: wound stabilization, space creation and maintenance, protection of the underlying blood clot, and the ability to exclude unwanted tissues or cells [13].

GBR is assumed to be achieved when the osteoprogenitor cells are exclusively allowed to repopulate the bone defect site by preventing the entry of non-osteogenic tissues. Eligali [14] has classified GBR membranes according to the type of biomaterial used and included synthetic polymers, natural polymers, metals and inorganic compounds. Synthetic polymers include polytetrafluoroethylene, aliphatic polyesters like polycaprolactone, polyglycolic acid, and polylactic acid membranes. Natural polymers include collagen membrane, chitosan and alginate. Inorganic compounds include calcium sulphate and calcium phosphate (hydroxyapatite). Requirements for an ideal GBR membrane include rigidity so as to withstand the compression of overlying soft tissue, cell occlusiveness, space maintenance, tissue integration, adequate mechanical and physical properties [14].

Thomas [15] stated that although these resorbable and non-resorbable membranes act as physical barriers they possess many bio-functional limitations and the ideal membrane for use in periodontal regenerative therapy has yet to be developed. For better bioactivity, cell proliferation and adhesion it is necessary to combine it with other bioactive materials and nanoparticles like titanium and titanium alloy, cobalt chromium alloy and inorganic compounds include calcium sulphate and calcium phosphate (hydroxyapatite) etc. [16]. Joanna Smardz [17] stated that due to the wide use of polymeric membranes and the constant development in the field of dentistry, there is a need to create a collective study, in which new trends in membrane modifications are presented.

SOURCES

Nonresorbable membranes including titanium foils and expanded polytetrafluoroethylene (e- PTFE) with or without titanium reinforcement were evaluated. These biomaterials are biocompatible, inert and do not elicit immunological reactions that may interfere with the regenerative process. The titanium frame, when adopted generates a mechanical support for the soft tissues over the defect to be regenerated and prevents the collapse of the mucosa into the wound area. Space provision plays a fundamental role in both periodontal and b one regeneration [18]. Studies demonstrated that the use of titanium reinforced membranes alone or with a filling material results in significant bone formation even in large non-space-maintaining implant dehiscence [19,20]. Main disadvantage of nonresorbable membranes is that there is a need for an additional surgery for them to be removed. Another drawback of e- PTFE membranes was related to the unfavorable outcomes achieved when membrane exposure occurred including infection and limited bone regeneration [19].

In order to eliminate the drawbacks of nonresorbable membranes, several types of biodegradable membranes were introduced. Originally, resorbable membranes were mainly based on polyesters (polyglycolic acid (PGA), polylactic acid (PLA)) and tissue-derived collagens [20]. Polymeric resorbable membranes remain stable for about 14 days and then gradually lose their structural and mechanical properties within 30 days [21]. Polymeric membranes also showed limited biocompatibility. Collagen membranes are more biocompatible than polymeric membranes, but they showed poor mechanical properties compared to nonresorbable membranes [22]. Clinical and preclinical studies compared resorbable and nonresorbable membranes in terms of defect fill in bone regenerative procedures and systematic reviews evaluated their use in both GTR and GBR and concluded that with respect to horizontal bone fill, resorbable barrier membranes showed better results, and therefore, long-term studies with larger sample size and more advanced techniques for the assessment of changes in the parameters should be carried out for the results to be more conclusive [23-27].

In cases where membranes were not exposed, defect fill

was greater when using e- PTFE than resorbable membranes. At the time, these results were explained considering the following features of e-PTFE membranes: (i) better space provision, (ii) controlled time of the barrier function, (iii) absence of inflammatory resorption that negatively influences tissue regeneration and (iv) better surgical protocols with e-PTFE membranes originating from a longer experience. Minabe [13] demonstrated the stability of periodontal tissues regenerated with resorbable and nonresorbable membranes at 10 years after treatment. Khor [28] claimed that both collagen and e-PTFE membranes are suitable treatment options for GBR applications, but membrane fixation is fundamental in achieving a successful outcome of the treatment. Pitaru [29] demonstrated that vertical bone regeneration obtained both with resorbable membrane supported by osteosynthesis plates and nonresorbable titanium reinforced e-PTFE membrane can be successfully maintained up to 3 years after implant loading [30]. The clinician must consider that e-PTFE membranes previously evaluated are no longer available in the market. Several advances in resorbable membranes technology have been introduced including cross-linked collagen membranes with longer resorption time and better biomechanical properties when compared to non-crosslinked membranes [31]. The use of resorbable membranes is now sustained by a large evidence and increased experience levels given the widespread use of these products in recent years. However, in a human study, intrabony defects were treated with GTR and resorbable collagen membranes and histological evaluations revealed the formation of long junctional epithelium above newly formed cementum and periodontal ligament. Furthermore, the study observed that filling material was mostly embedded in connective tissue, without any evidence of bone regeneration [32]. More recently, high density PTFE (d-PTFE) membrane is being more closely evaluated. Because of its smaller pore size compared to e-PTFE membranes, d-PTFE seems to withstand exposure to bacteria from the oral cavity, reducing the drawbacks of membrane exposure even when using this non-resorbablebarrier [33].

CHARACTERIZATION

Several techniques for scaffold fabrication have been reported in the literature, e.g., salt or sugar leaching foam replication methods, thermally induced phase separation, electrospinning for nano-fibrous structures microsphere emulsification sintering, computer aided rapid prototyping techniques, textile, biomimetic approach foam coating methods. These methods were done so as to optimize the properties, structure and mechanical integrity of scaffolds. The incorporation and design of nano-topographic features on scaffold surface architecture for mimicking the nanostructure of natural bone, is becoming a significant area in bone tissue engineering research [34].

Scanning Electron Microscopy (SEM), Attenuated total reflectance-Fourier transform infrared spectroscopy (FTIR-ATR), X-ray Diffraction (XRD), Confocal microscopy is used to examine the morphological characterization of GTR and GBR membranes.

CLINICAL APPLICATIONS OF GTR AND GBR MEMBRANES

Karring [16] stated that the strategy to isolate the periodontal defect with a mat- like material (resorbable or non-resorbable) that will function as a physical barrier to avoid gingival cell invasion led to the development of guided tissue regeneration membranes. Selecting ideal biomaterial for guided tissue regeneration membranes include following requirements: wound stabilization, space creation and maintenance, protection of the underlying blood clot, and the ability to exclude unwanted tissues or cells [35-37].

Osseous defects can be managed with simultaneous implant placement in implant dehiscence or fenestration defect in which defects are corrected using barrier membranes [6]. Guided bone regeneration is a successful, well-documented and widely used procedure for treatment of alveolar bone defects in conjunction with implant treatment. A systematic review reported 95% implant survival after a horizontal or vertical GBR procedure [38].

Clinical studies demonstrate that GBR is predictable and successful for horizontal defect augmentation and in most instances, this can be achieved using either non-resorbable or resorbable membranes. Clinical studies have also used titanium-reinforced ePTFE membrane, in combination with bone-filling materials, to enhance vertical bone augmentation. Although non-resorbable membranes have been more commonly used for vertical bone defects, recent clinical studies showed promising results with the use of resorbable collagen-based membranes. Taken together, clinical studies, systematic reviews and meta - analyses show successful outcomes with GBR procedures for alveolar bone augmentation and implant placement. However, some clinical situations remain challenging, especially in cases of vertical and advanced horizontal alveolar bone atrophy [39].

BIOACTIVE NANOPARTICLES AND BIOACTIVE MOLECULES

Biomaterials used for bone replacement grafts developing new and healthy bone tissue should meet certain specific requirements:

Biocompatibility: Interaction between the material and the tissues should not affect the surrounding tissues adversely, safety of the patient and the intended healing result. Ideally, biomaterials should be bioactive inherently for promoting bone regeneration process (e.g., surface characteristics and ion release).

Porosity: Adequate pore size, morphology and inter-connectivity is needed for allowing diffusion throughout

whole scaffold of nutrients, bone cells and exchange of waste products. It is important to distinguish between macro-porosity and micro-porosity (Hutmacher, 2000). Micro-porosity is defined as pores ≤10 μm for improving cell adhesion, for allowing fluids and nutrients flow (permeability)and thereby enhancing the bioactivity. Macro-porosity is defined as pores ≥100 μm for allowing angiogenesis and bone cell ingrowth, thereby mimicking porosity of trabecular bone, which has a mean value 250 μm, although it is highly variable. Inter-connectivity connection between pores is also important property for allowing for vascularization and bone ingrowth permeability.

Osteoconductivity/Osteoinductivity: All biomaterials for bone regeneration should allow bone growth directly in contact with biomaterial surface from the surrounding bone (*osteoconduction*), but ideally it should also be able for promoting *osteoinduction* (Albrektsson & Johansson, 2001). An *osteoinductive* biomaterial should be capable first of recruiting mesenchymal-type osteoprogenitor cells. Secondly, it should be capable of transformation of an undifferentiated mesenchymal cell into a mature bone-forming osteoblast. Lastly, it should be capable of inducing ectopic bone formation ingrowth when implanted into the extra-skeletal locations. This capacity may be related to its surface properties and microporosity.

Surface properties: Surface topography at the micro and nano level as well as the surface physico-chemistry are important characteristics for extracellular matrix deposition, cell adhesion protein adsorption, differentiation, migration and bone formation finally.

Biodegradability: It is the capacity of the biomaterial to bio-absorb during the remodeling and tissue healing process. An ideal bone graft substitute is expected to be fully replaced by bone, at a predictable absorption rate preferably, without interfering with the healing and regeneration process and without losing tissue volume. For biomaterials with a slow bio- absorbability rate, these should assure a process of new bone formation with sufficient volume in biomaterial contact.

Mechanical properties: Elasticity and compressive strength should be high enough to absorb the load from surrounding soft and hard tissues in non-contained defects. Ideally, the elasticity and compressive strength of the biomaterial should be at least those of the natural bone at their generation site. These mechanical properties are influenced by pore size and morphology also.

Antigenicity: The inherent biomaterial properties (e.g., porosity and surface) should promote appropriate vascularization of the graft volume and angiogenesis [40].

Handling: Biomaterial should be dimensionally stable and cohesive, and easy for chairside use for adapting to the defect. When using in non-contained defects, it should allow build up three-dimensionally. Biomaterials for bone regeneration in craniomaxillofacial region are usually available in the form of blocks or granules. Depending on the clinical needs, it is desirable to have a wide variation of sizes and forms, ranging from 0.1 to 2.0 mm in the particulate form (Haugen, Lyngstadaas, Rossi, & Perale, 2019). For some indications, an injectable mode of application is desired to fill the defect volume through its plasticity.

Manufacturing processes: Biomaterial must be provided with certification or documentation of appropriate sterilization and manufacturing processes and assure reduced production costs and long shelf time [41].

ADVANTAGES AND LIMITATIONS OF THE CURRENTLY USED BONE GRAFTS

Autologous bone

Autologous bone is not a biomaterial per se, but is considered as the gold standard graft material for bone regeneration and has the following advantages: it contains patient's own cells, bimolecular and growth factors needed for osteogenesis, it has the highest degree of biocompatibility, matched mechanical properties, biological safety and scaffolding effect [42]. Limitations include autologous grafts which may need a second surgical site for its harvesting, which indeed increases patient's morbidity, discomfort or pain and other complications related to increased invasiveness and surgical time. It has been reported that the resorption of these bone replacement grafts is higher, and their resorption rate is not predictable. Depending on the graft source (cortical vs. cancellous bone) the vascularization may be slowed down, mainly in highly cortical bone grafts. It has also limitations in terms of volume availability, mainly when harvesting from sources in intra-oral region and the resulting grafts, mainly in a block form may be difficult for adaptation to the anatomy of the defect. The application of particulate dentin has been recently suggested as another autologous source for socket site preservation and minor ridge augmentation. However, there is no clinical documentation for substantiating its clinical use [43-45].

Allogenic

There are different ways of allogenic bone replacement grafts processing (fresh frozen and freeze dried), which may change their biological properties. These allografts are produced as particulate or blocks. It has the advantage of providing mechanical properties which are similar as the autologous bone and it may contain the collagenous matrix and proteins of natural bone, even though it lacks viable cells. Similarly, handling properties are comparable to autologous bone, even though it reduced surgical time needed for its implantation; in addition to increased availability are clear advantages, when compared with autologous bone. Its biological safety because of disease transmission and potential unwanted immune reactions are

clear disadvantages. Furthermore, the sources of donor material are heterogenous, which might influence their biological activity and similarly, resorption rates are greatly variable. Other drawbacks could be impairment to achieve vascularization of the grafted site [46].

Xenogeneic

Deproteinized bovine bone mineral (DBBM) is the biomaterial with the most documentation for bone grafting in the scientific literature. Its main advantages are that since it is derived from both natural cancellous and cortical bone its geometric structure and architecture may resemble bone, although it is dependent on the tissue source and manufacturing process. Its slow bio-absorbability might be clinical advantage for preservation of the augmented bone volume. With regard to limitations, it lacks biological components thereby limiting its biological activity. Similar to allogenic materials, the use of it implies a potential biological risk of disease transmission (e.g., prions and retroviruses) and/or immunogenic host-tissue response, even though these risks can be diminished through the manufacturing process (deproteinization). In spite of this inherent risk, however, transmission of bovine spongiform encephalitis (BSE) has yet not been reported to be associated with the implantation of this biomaterial. Mechanical properties (brittleness) may vary depending on the manufacturing process and source. Since these biomaterials are available mainly for use in particulate form, they may have limitation in large defect regeneration interventions [47].

Synthetic Bio Ceramics

Calcium phosphate, calcium sulphate, bioactive glass and combinations are the most commonly used bio ceramics available at present. Their main advantage is the controlled manufacturing process which may assure biodegradability, biocompatibility and similarity in structure and inorganic composition to natural bone minerals. The most commonly investigated calcium phosphate bone graft substitutes are hydroxyapatite (HA), β-tricalcium phosphate (β-TCP) and their combination, also called biphasic calcium phosphate (BCF) [50]. Bio ceramics have shown osteoinductive properties through stimulation of inorganic matrix deposition, osteoblast growth and bone promotion and osteoblast differentiation. By sintering temperature and modulating their chemical composition, their degradation time and bioactivity can be controlled to a certain extent [49].

Calcium Phosphates

Calcium phosphates are frequently used in dental and bone tissue engineering applications due to their compositional affinity with mineral phase of natural bone. In general, these materials tend to induce biological response which is very similar to that generated during bone remodeling. Since calcium phosphate grafts are chemically similar to the natural bone, their products of degradation are non-toxic and can be metabolized naturally by mammals. Implantable calcium phosphates are available as scaffolds, granules, solid pieces, 3D porous and pastes or cements. In reconstructive periodontal strategies, tricalcium phosphates, hydroxyapatite (HA), and their combinations are recognized as the most commonly used calcium phosphates [50].

HA (Ca_{10} $(PO_4)_6(OH)_2$) is one of the most abundant inorganic components of natural bone (around 65% of its inorganic phase) and in clinical practice it has a lot of applications as a bone filler. Lot of studies have been implemented on the interaction between bone and HA implants. Direct chemical bond was found between HA graft and osseous tissue, which gave rise to a kind of bone matrix formation on implant surface. This newly-formed matrix consist of both well-organized network of collagen fibers and globular mineral deposits which would indeed enhance the interfacial bonding. By attaching on the HA surface osteoblasts can start mineralized osteoid formation, which then matures into fully mineralized bone. Apatite crystals might also incorporate other ions like carbonate groups which appear on the implanted HA grafts surface and thereby exhibits a nano-crystalline morphology mimicking the biological apatite of alveolar bone. HA is osteoconductive but its disadvantage of having slow degradation rate limits its use alone [51-54].

Tricalcium phosphate (TCP) materials interest has increased rapidly in recent years. The degree of solubility depends mainly on the Ca-to-P ratio (the rate of dissolution increases with decreasing Ca/P ratio) and also on the crystallographic structure, which may lead to following progression: HA < β-TCP ≪ α-TCP) [54].

The most attractive TCP phase in biomedicine is the β one, which exhibit good osteoconductive properties and biocompatibility. However, β-TCP exhibits degradation kinetics which is comparable to the rate of new bone growth and regenerative properties similar to autologous bone grafts. β-TCP is used as implant material in low-bearing applications and in particle form due to its poor mechanical properties [54].

A suitable grafting material for periodontal tissue engineering should be osteoconductive and must be able to sustain the load applied on the defect site as new bone grows, and should safely dissolve without producing any toxic ionic species for the surrounding tissue. The balance between HA and β-TCP is a crucial point in order to obtain adequate mechanical suitable degradation kinetics, strength and osteointegration in biphasic calcium-phosphate ceramics. Several studies were performed for assessing the best HA-to-β-TCP ratio, but the results were difficult to compare as many variables affect the conclusions from one to another group of researchers. The dissolution rate of pure β-TCP and the totally absent osteoclastic activity on the pure HA suggest that the use of a combination of β-TCP and HA

(as a biphasic material) provides a proper condition for osteoclasts to act more naturally [55-57]. As new bone deposition by osteoblasts is related strongly with osteoclastic resorption during bone remodeling, biphasic calcium phosphates create a surface similar to that of the native bone, and thereby the dissolution/precipitation process which occurs during the osteoclastic activity favors the formation of a chemical bond between bone apatite and similar apatite formed on the ceramic surface. By changing the proportion between HA and β-TCP, it is possible to control the degradation rate of the bone graft and bone formation [58]. Morra [59] recently developed a biphasic granulate bone filler with a HA/β-TCP weight ratio of 75/25; after being implanted in rabbits, this graft exhibited an excellent new bone formation without any inflammatory response. The mechanical properties were found to be depended not only on the composition but also on the geometry (e.g., the presence of macropores) and process parameters (i.e., sintering temperature and time), thereby opening a broad range of possibilities for optimizing the properties of these bone grafts for application in periodontal tissue engineering.

Bioactive Glasses

In bone tissue engineering, a bioactive material undergoes specific surface reactions *in vitro* and *in vivo*, which leads to the formation of a HA-like layer that allows a strong bond between host bone and grafting material. Recent studies have revealed that bioactive glasses are osteoinductive materials and they can induce osteoprogenitor cells for migrating into the structure of the graft and thereby promoting cell differentiation and gene expression of undifferentiated cells and these exceptional properties are due to the release of therapeutic ionic species, mainly silicate and calcium ions, which stimulate bone cells towards regeneration and self-repair [60].

Most commonly-used bioactive glass with a history of 50-years is the well-known 45S5 Bioglass which exhibits a relatively low SiO_2 content, high Na_2O, and CaO content, and high CaO/P_2O_5 ratio for favoring the reactivity with biological fluids. This composition promotes the formation of a bone-like nano-crystalline HA layer on the surface of the glass, which can firmly bond to the host bone. 45S5 glass particulate, commercially marketed as PerioGlas in dental applications, was able to inhibit the down-growth of epithelial cells and was able to promote the regeneration of alveolar bone. Addition of bioactive glass particles to the autologous material can be used in large intrabony defects, where large amount of grafting material high mechanical properties is needed. Recent studies in small animal models have proposed the use of borosilicate glasses for bone regeneration as they are characterized by faster apatite-forming and bone-regenerative abilities compared to silicate glasses [60].

Calcium Sulfate

Calcium sulfate, also known as 'plaster of Paris', was earlier used as a tissue augmentation material to fill cavities caused by tuberculosis. Since then, it has been widely used in orthopedics and dentistry in order to fill the bony defects. Three forms of calcium sulfate exist depending on the number of water molecules inside their crystalline structure, which includes anhydrate, dehydrate, and hemihydrates. Calcium sulfate leaves behind calcium phosphate deposits after being completely degraded in biological fluids thereby stimulating bone growth. However, porosity and hygroscopic properties of calcium sulfate are the key factors which allows the adsorption and infiltration of platelets for stimulating the bone and new blood vessel formation (i.e., angiogenesis). No adverse reaction (e.g., immunogenicity) have been reported regarding by-products of calcium sulfate. Calcium sulfate is preferably used in the forms of moldable paste or putty and safely used in filling periodontal defects. In order to overcome the problems associated with fast resorption, calcium sulfate is combined with other materials, such as calcium phosphates, thereby achieving a more stable structure and a finer control on the resorption kinetics. Other approaches include the production of biphasic calcium sulfate, in which dehydrate and hemihydrate types are mixed for decreasing the dissolution rate and for making a rigid matrix post-implantation. The primary uses of calcium sulfate and its composites in dentistry and maxillofacial surgery are in the field of injectable bone fillers for alveolar bone regeneration in small periodontal defects and for sinus augmentation [61]. Main disadvantage of calcium sulphate is associated with limited mechanical properties (loadbearing resistance) and unpredictable bio-absorption rates. They are mainly delivered as particulates, which may limit their use in large bone defects. In order to improve their mechanical therapies (brittleness), bio ceramics is mixed with polymers developing composite materials [62].

Synthetic Polymers

Synthetic polymers which are used as biomaterials for bone tissue regeneration includes aliphatic polyesters like PLA, poly (ε-caprolactone) (PCL), and PGA and their copolymers and derivatives. They have the advantage that their manufacture is controllable and tunable in terms of adjusting their physiochemical structure, porosity, and also their biodegradability and shape, size and biomechanical properties can be customized. The major limitation is that they do not demonstrate osteoconductivity and thus, their use as bone replacement grafts requires combination with bio ceramics as composite materials or they can be functionalized. Their process of bio-absorbability causes release of acid compounds which may interfere with wound healing, Furthermore, the bio-absorbability of synthetic polymers is highly variable, which impairs their mechanical strength *in vivo* [63].

CHEMICALLY MODIFIED COLLAGEN

Number of different methods of physical/chemical cross-linking have been developed, in order to slow down the bio-absorption process of collagen membranes which may also enhance its mechanical properties. Although chemical cross-linking has caused an improvement in collagen stability, release of chemicals residues (e.g., amides or aldehydes) have been associated with severe inflammation at the implantation site. Generally, the predictability of the collagen membrane not only depends on the origin of the collagen material but also its manufacturing process and preparation [64].

Chitosan

Chitosan is a natural linear polysaccharide derived from chitin and is commonly extracted from the crustaceous exoskeleton which is composed of randomly distributed β-(1,4)-glucosamine and N-acetyl-D-glucosamine. White mushrooms are the other sources of chitosan which can be preferred for eliminating any animal-related immunogenic and ethical issues; however, its extraction process is expensive, and the yield is lower. The widespread use of Chitosan in bone tissue engineering applications and pharmaceutics is due to its biocompatibility, antibacterial activity, and the ability to promote cell adhesion, proliferation, and differentiation. Furthermore, Chitosan has a backbone which is similar to that of glycosaminoglycans, the major components of bone ECM. After being implanted in patients with periodontitis, Chitosan could reduce gingival inflammation due to its antimicrobial properties [65].

Other biomedical applications include combination of soft Chitosan with stiff, high-strength bioceramic to produce porous foams or composite pastes. Chitosan exhibits a poly-cationic nature which allows the creation of ionic interactions with other poly-anionic materials, and thereby generating the so-called polyelectrolyte Hydrogel. These properties are mainly used to produce materials for controlled drug release, especially for intestinal tract use. The ionic bond is pH-sensitive and, therefore, the drug release rate depends on the pH of the organ [66].

Pectin

Pectin is a natural anionic polysaccharide which is abundantly present in citrus cell walls and apple peel by-products. It consists of poly (D-galacturonic acid) chain with partly- methoxylated carboxylic groups. The ionic crosslinking of pectin carboxylic groups is achieved by calcium ions to form the so-called “egg box” structure, in which a divalent cation (Ca^{2+}) is bonded with different carboxylic anions. The major disadvantage of pectin is its fast solubility in aqueous media, which causes rapid dissolution and when used as a drug carrier, there occurs a burst release of the therapeutic molecules. For overcoming this problem, studies have been conducted to combine pectin with other materials, such as Chitosan, for increasing the chemical resistance in water. Pectin has also been used in combination with calcium phosphate particles, for mimicking the ECM and guide cell proliferation, or it can also be applied as a surface coating in different biomedical applications, such as antiadhesive surgical meshes [67].

Hyaluronic Acid

Hyaluronic acid (or Hyaluronic) is a highly-attractive material for periodontal tissue engineering and is one of the natural glycosaminoglycans contained in the ECM of connective tissues, which exhibit excellent potential for making scaffolds for tissue regeneration. Hyaluronic acid is a linear polysaccharide and, in dentistry, it elicited anti-inflammatory and antibacterial effects in the treatment of periodontal diseases. The repeating unit of Hyaluronic acid consists of d-glucuronic acid bonded to N-acetyl-d-glucosamine. Hyaluronan exhibits hygroscopic and viscoelastic properties, which allows the material for absorbing huge amount of water thereby maintaining conformational stiffness for filling the defect space, thereby protecting the exposed tissue surfaces in periodontal surgery. Hyaluronic acid can act as a stable barrier against the penetration of viruses and bacteria and can elicit a bacteriostatic effect, which helps to avoid the contamination of surgical wounds by foreign pathogens and also helps to reduce the risk of postoperative infections, thereby promoting a more predictable regeneration [68].

BIOACTIVE MOLECULES FOR ALVEOLAR BONE REGENERATION

Endogen

Amelogenine form the major proteinic component of extracellular matrix proteins with high affinity for hydroxyapatite and dental root surface. During the process of odontogenesis and development of tooth attachment apparatus, these proteins adsorb on the root surface and leads to the formation of acellular cementum. In 1997, a purified acid extract of enamel matrix proteins (Emdogain, EMD) was incorporated in a human experimental defect, and the formation of new acellular extrinsic fiber cementum was assessed. In a human histologic sample of a tooth treated for gingival recession with connective tissue graft (CTG) + EMD there occurred formation of woven bone and connective tissue anchored in the new cementum. *In vitro* studies were conducted to assess how enamel matrix derivatives stimulated PDL fibroblast and osteoblast proliferation differentiation [69].

Several trials were performed to assess the efficacy of enamel matrix derivative (EMD) on reducing pocket probing depth of infrabony and furcation defects and in the treatment of gingival recessions. A recent systematic review was conducted to evaluate the benefits of additional use of EMD in periodontal regenerative procedures and the authors stated that the use of EMD in the treatment of

infrabony defects is superior in terms of CAL gain as compared to open flap debridement, placebo or root conditioning with 24% EDTA, and as effective as resorbable membranes. In the treatment of gingival recession, the coronally advanced flap technique (CAF) + EMD was found to be more effective than CAF alone, but no differences were found between the CAF + EMD group and the CAF + CTG. In the treatment of furcation defects, EMD gave more reduction in horizontal furcation defect depth than the use of a resorbable membrane. Long-term clinical study confirmed that the clinical improvements obtained with the EMD use can be maintained over a period of 10-15 years [70].

Studies on osteopromotive EMD effects showed a more extended range of clinical applications of this product in dental practice than the only tooth supporting regenerative therapy including: the bone and peri-implant bone regeneration. Preclinical studies were performed to evaluate the effects of GBR in combination with or without deproteinized bovine bone mineral (DBBM) and/or an enamel matrix derivative (EMD) on bone healing and regeneration and the authors stated that the use of EMD does not positively affect the amount of new bone formation and that the predictability of bone formation in critical-size defects depends mainly on the presence or absence of barrier membranes (GBR). The combined use with deproteinized bovine bone mineral and/or enamel matrix proteins did not significantly improve the potential for complete healing provided by the GBR procedure. EMD's beneficial effects includes formation of periodontal ligament and cementum, while its impact on new bone regeneration is found to be limited [71].

Growth factors

Recently recombinant Growth Factors have been introduced which includes platelet-derived growth factor (PDGF-BB), transforming growth factor-beta1 (TGF-β1), insulin-like growth factor-1 (IGF-1), vascular endothelial growth factor (VEGF), endothelial cell growth factor (ECGF), fibroblast growth factor-2 (FGF-2), and bone morphogenetic proteins (BMPs). By using recombinant GF purified solutions with higher concentrations of a single GF or combinations of GFs can be achieved [72].

Bone morphogenic proteins (BMPs) induce the differentiation of the host stem cells into bone forming cells (osteoinduction). RhBMP-2 absorbed in a collagen sponge was evaluated for alveolar ridge preservation after tooth extraction in both the posterior segments as well as in more challenging defects. RhBMP-2 and RhBMP-7 seem to have great potential for GBR applications, although rhBMP-12 may be more appropriate for GTR. RhPDGF-BB has been accepted by the FDA for regeneration of bone and PDL elements in procedures like guided tissue regeneration. Good results were shown by using this growth factor both in GTR as well as in GBR such as socket grafting, localized grafting procedures, maxillary sinus augmentation, and vertical ridge augmentation [73].

FGF-2 has been extensively used to evaluate periodontal applications, Fibroblast growth factor (FGF)-2 displayed potent angiogenic activity and mitogenic ability on mesenchymal cells especially on PDL cells and decreased alkaline phosphatase activity. Exogenous FGF-2 may act differently on PDL cells and gingival epithelial cells *in vivo* in terms of proliferative response, thereby blocking epithelial downgrowth and stimulating PDL cell growth [74,75]. Other studies have evaluated the use in periodontal therapy of platelet-derived growth factor (PDGF) in combination with insulin-like growth factor (IGF)-I, bone morphogenetic protein (BMP)-2, transforming growth factor (TGF)-b, osteogenic protein (OP)-1, and brain-derived neurotrophic factor (BDNF). Use of recombinant growth factors in GTR and GBR showed interesting results because of the inflammatory environment in surgical areas, and it was found that their presence in the wound area seemed confined to the first few hours [76].

PDGF is the main growth factor involved in wound healing, and there have been a lot of *in vitro* and *in vivo* studies showing its ability to enhance the proliferation and migration of periodontal ligament cells. PDGF is naturally made by the conjugation of polypeptides of growth factor-BB and growth factor-AA, which was encoded by two different genes. It has been found that all isoforms have an effect on cell proliferation *in vitro*. PDGF has a chemotactic effect, which leads to collagen synthesis, and stimulates hyaluronate synthesis by gingival fibroblasts and fibroblast proliferation. Furthermore, if added to a culture with osteoblast-like cells, PDGF can cause the regulation of ALP and osteocalcin expression. Lynch et al. applied PDGF in combination with the insulin-like growth factor-1 (IGF-1) in dogs, and the results showed great effectiveness in periodontal regeneration. Also, the clinical trial results revealed that the synergistic effect of these two growth factors could lead to the stimulation of bone regeneration in periodontal defects in humans, too. When used alone, PDGF can also significantly stimulate the formation of new cementum and the production of collagen [77].

Bone Morphogenetic Protein

Bone Morphogenetic Protein (rhBMP-2) (at different dosages) significantly promotes bone regeneration in critical and sub- critical size bone defects and denovo bone formation regardless of the carrier adopted. rhBMP-2 promotes ridge augmentation in chronic and combined defects and enhances ridge preservation. Conflicting results were reported regarding the benefits in peri-implant circumferential defects and sinus augmentation. As a carrier, the absorbable collagen sponge (ACS) can be used successfully with or without space- providing materials. For clinical applications, ACS carrier was impregnated with rhBMP-2 which was approved by Food and Drug

Administration for ridge preservation and sinus augmentation. Most of the clinical studies have employed BMP-2/ACS, although a combination of rhBMP-2 with different grafts have also been suggested. Clinical studies suggested 1.50 mg/ml as the optimal dosage for ridge preservation and a range between 1.05 and 4.2 mg/ml for ridge augmentation procedures, while in some studies on sinus augmentation high supra-physiological doses up to 48 mg of BMP-2 per subject have been reported [78].

According to 3 RCTs, rhBMP-2/ACS were combined with osteoconductive grafts and a titanium mesh for ridge augmentation was found to be comparable to autologous bone and titanium mesh or deproteinized bovine bone mineral based on radiographic/histological outcomes. In a recent RCT of 4 months, the use of autologous block grafts was found to be superior in terms of amounts of mineralized tissue when compared to DBBM block grafts loaded with BMP-2. Three RCTs used BMP-2 combined with ACS or other carriers for ridge preservation. The use of rhBMP-2 in regeneration of bone defects following implant placement is scarce. The existing RCTs suggest that there is a similar beneficial effect of rhBMP-2/ACS when compared to commercially available bone grafting materials for socket preservation and ridge augmentation. Currently, this material was not approved in Europe for clinical use in oral and maxillofacial applications [79].

GENE THERAPY APPROACH IN PERIODONTOLOGY

The use of high dosage of bioactive molecules is needed to promote tissue regeneration, which could lead to unpredictable reactions and side effects; therefore, an alternative approach to the local release of growth factors is the use of gene therapy for periodontal regeneration. Gene therapy involves the insertion of the genes of interest into an individual's cells for obtaining the desired functions, i.e., in most cases, upregulation of the expression of a specific growth factor. Two main strategies have been developed including(i) the *in vivo* technique, in which the gene vector is directly inserted into the target site, and (ii) ex vivo technique, in which selected cells are harvested, expanded, genetically transduced, and eventually re- implanted. Gene therapy has been applied for the upregulating the expression of PDGF and BMPs. In the *in vivo* technique the gene of interest is directly delivered in the body, thereby altering the normal expression of the target cells. On the contrary, the ex vivo technique involves the use of an adenovirus vector for introducing the genetic material into the target cells that have been harvested by a biopsy; eventually, transfected cells are re-implanted in the periodontal defect [80].

PERIODONTAL REGENERATION BY PERIODONTAL LIGAMENT STEM CELL SHEET

Mesenchymal stem cells are widely used cell type for cell-based treatment because of their characteristics such as multi-differentiation capacity, immunomodulation, anti-apoptosis, angiogenesis, and cell recruitment. Besides these MSC characteristics, PDLSCs possess a potential to form cementum, and this characteristic of PDLSCs has stimulated the researchers to examine periodontal regeneration by transplantation of PDLSCs. Cell sheet engineering isa unique tissue engineering method for obtaining cells in a sheet format, which allows collection of the cell sheet without destruction of extracellular matrix components secreted from cells. The transplantation of PDLSCs induces regeneration of periodontal tissues, has gained wide acceptance. PDLSC transplantation is now considered one of the promising approaches for periodontal tissue regeneration [81].

BARRIER MEMBRANES WITH ANTIMICROBIAL ACTIVITY

The bacteria found on GTR membranes includes Gram-positive bacteria as well as periodontal pathogens. Membrane bacterial count is associated positively with gingival recession and negatively with clinical attachment gain. Usually, a systemic antibiotic is prescribed after a GTR operation to reduce bacterial contamination and to prevent wound infection, but the results are not predictable [82].

The incorporation of amoxicillin or tetracycline into various GTR membranes enhances the attachment of periodontal ligament cells in the presence of the oral pathogens' streptococcus mutants and aggregatibacter actinomycetemcomitans. Tetracyclines are also advocated as useful adjuncts in periodontal treatment. Incorporation of 25% doxycycline into a GTR membrane, composing of polyglycolic acid and polylactic acid, seemed to have a beneficial effect on periodontal bone regeneration in dogs. When clinically applied, tetracycline-loaded expanded polytetrafluoroethylene (ePTFE) membranes reduced bacterial contamination and increases clinical attachment gain. This proven efficacy is not only due to their antimicrobial actions but also to their recently recognized non antibacterial properties, which includes the anti-collagenolytic, anti-inflammatory, osteoclast inhibitory, fibroblast stimulatory properties. Tetracyclines have prolonged the degradation time of collagen membranes, this property can be made to use in certain clinical situations where it is desirable to retain the membrane fora prolonged duration of time [83].

RECOMMENDATION FOR FUTURE RESEARCH

The future of bone regeneration probably entails the manufacture of personalized biomaterials from 3-D digital data which is obtained from patients. Additive manufacturing (e.g., 3 -D printing) of different biomaterials (e.g., bio ceramics) allows rapid production of these customized scaffolds that will perfectly fit the bone defect anatomy. The addition of synthetic polymers in composite

biomaterials design may mechanically reinforce these 3D constructed biomaterials. Also, the addition of cells (bio-printing) may add biological activity to the 3-D printed constructs. Future biomaterials must have optimized surface characteristics, pore size and interconnection. These characteristics should be adjusted to control their bio-absorbability, promote osteoinduction and ensure ideal mechanical properties [84].

Biomimetic biomaterials should be developed at ambient temperatures through hydrolysis and precipitation of calcium deficient apatite, which will result in similar composition and crystallinity as natural bone. These biomaterials must be completely be replaced by new bone through controlled processes of bio-absorbability and osteoinduction. There is a need for standardized and validated pre-clinical models, by the use of small animal models for screening and large-animal models for comparing new biomaterials using established standards [85]. In concordance with the ARRIVE guidelines for reducing animal research, there is a need for standardized pre-clinical models, such as in silico modelling and ex vivo tissue engineering testing to reduce animal research [86].

CONCLUSION

Periodontium refers to the specialized tissues which surrounds and supports the teeth, by maintaining them in the maxillary and mandibular bones. Tooth loss has been a possible consequence of trauma or periodontal disease, such as gingivitis, periodontitis, or tissue decay. The scope of periodontal tissue engineering is to regenerate the tooth's supporting tissue by a combination of proper biomaterials, which stimulates cells and signaling molecules to produce a new healthy tissue [87]. Many advances have been made in the last decade for the regeneration of complex periodontal and alveolar bone defects. Research efforts in polymeric and ceramic scaffolding systems for cell, protein, drug, and gene delivery lead to the development of a complex and often multifunctional implants with a predictable response [88].

In the research world, there is still some debate regarding best treatment modality for obtaining periodontal regeneration. Some groups advocated the use of bone replacement grafts alone, while others suggested that a guided tissue membrane (GTR) alone might be sufficient on resorbable membranes. In general, case selection is very important to the success of regenerative strategies, which might explain some of the inconsistencies in the literature. Factors which affect clinical success can be related to the specific patient, specific disease and healing categories. The success of a surgical procedure involves the use of a bone grafting material, a GTR membrane or else a combination of both which depends on good plaque control, compliance, non-smoking, anti-infective therapy, and systemic health. During surgical procedures, there may be certain additional variables which could affect the results of the regeneration process, such as the possible infection of the implanted material, which could cause peri-prosthetic infection [89].

In summary, periodontal regeneration still remains a partially unmet challenge. The incorporation of growth factors in periodontal biomaterials and scaffolds is indeed a valuable strategy for improving regeneration, but biomolecules are typically expensive, which makes them accessible just to a minority of patients, and can elicit unpredictable side effects even at low dosage. Gene therapy has opened up new horizons for treating congenital dental diseases in individuals and their offspring. Loading and controlled release of therapeutic ions, can stimulate the genes of cells towards paths of targeted tissue regeneration and self-repair, which might be a highly-attractive alternative deserving investigation in the near future. Furthermore, the development of functionally-graded scaffolds for mimicking the composition and micro structural organization of the tissues to regenerate represent a key step towards the simultaneous healing of multiple periodontal tissues.

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