CHAPTER 5

Calcium Phosphate Ceramics in Periodontal Regeneration

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Abstract: Regenerative periodontal therapy aims to predictably restore the tooth's supporting periodontal tissues and should result in the formation of a new connective tissue attachment (*i.e.* new cementum with inserting periodontal ligament fibres) and new alveolar bone. This chapter aims to address the clinical application of bone grafts on periodontal regenerative approaches, with given relevance to the use of calcium phosphate ceramics. Furthermore, a clinical case is presented in which the regenerative capability of a glass-reinforced hydroxyapatite (Bonelike®) is thoroughly evaluated by clinical and tomographic measurements in the healing of a periodontal intrabony defect.

Keywords: Calcium phosphate materials; intrabony periodontal defects; periodontal regeneration.

1. PERIODONTAL REGENERATION

1.1. Periodontal Disease

Periodontal disease is an inflammatory condition, inherited or acquired, that affects the surrounding and supporting tissues of the teeth. It is characterized by a chronic oral bacterial infection that results in inflammation of the gums, which thus leads to the gradual destruction of periodontal tissues, including the alveolar

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bone that embraces teeth support [1]. Epidemiologic data estimates differ on the basis of the definition of the disease, its prevalence, severity, and rate of disease progression, and clearly fluctuates worldwide by race and geographic area, with older populations typically experiencing higher rates of periodontitis [2]. Estimates of the global prevalence of severe periodontal disease are broadly around 10-15%, although up to 90% of the population may be affected by some form of milder periodontal disease including gingivitis [2, 3].

In addition to age and race, other known risk factors for periodontal disease include gender, body mass index (obesity), tobacco consumption, stress and nutrition, as well as systemic conditions like diabetes, osteoporosis and neutrophilic dysfunctions [4-6]. Populations with limited access to dental healthcare and also those with a low socio-economic status may also be at an increased risk for the establishment and development of periodontal disease [5]. Furthermore, data of genetic polymorphisms associated with periodontal disease have been reported, noticeably those related to the interleukine-1 gene cluster [7].

Periodontal disease is a pathology with infectious etiology, caused broadly by anaerobic Gram-negative bacteria. Socransky *et al.* examined over 13,000 sub-gingival plaque samples from 185 adult subjects and used cluster analysis and community ordination techniques to group the identified bacterial species. Six closely associated clusters were consistently recognized and subsequently color-coded into their respective complexes, *i.e.* “Blue”, “Green”, “Yellow”, “Purple”, “Orange” and “Red”. The first 4 complexes were described to be early colonizers of the tooth surface and to form the conditioning biofilm before the proliferation of the more pathogenic “Orange” and “Red” complexes [8]. The “Red” complex was shown to be strongly related to pocket depth and bleeding on probing – clinical signals of periodontal disease. Further, it has been shown that during the biofilm maturation, organisms from the “Orange” complex are required for the further establishment and colonization of the “Red” complex. The presence of these two complexes, in particular the “Red” complex, has been shown to be strongly correlated with severe and advanced stages of periodontal disease. In the “Red” complex, *P. gingivalis*, *T. denticola* and *T. forsythia* are included, while the “Orange” complex includes *F. nucleatum/periodonticum* subspecies, *P. intermedia, Prevotella nigrescens, P. micros, C. rectus, Campylobacter gracilis,*

Campylobacter showae, Eubacterium nodatum and *Streptococcus constellatus* [8]. Interestingly, many of these bacteria also seem to be present at low levels in the dental plaque of healthy individuals [9]. Overall, periodontal disease evolution seems to be signaled by a shift in the makeup of the dental biofilm from largely aerobic Gram-positive bacteria to a pathogenic infectious state dominated by anaerobic Gram-negative organisms [9]. Thus, the onset of periodontal disease is not marked by the establishment of novel infectious strains, but rather by a shift in the dominant strains composing the dental plaque biofilm [10]. These microorganisms possess an array of virulence factors that enhance their infectivity and provide the ability to the organisms to multiply and persist in the periodontium [10]. While the etiology of periodontitis seems to be bacterial, it is becoming clear that the pathogenesis of the disease is mediated by the development of a coordinated host response that induces an inflammatory reaction, which is thus destructive to the periodontal tissues [11]. Initially, protective aspects of the host response include recruitment of specific cellular immune-relevant populations, production of protective antibodies, and possibly the release of anti-inflammatory cytokines (*e.g.*, transforming growth factor-β (TGF-β), interleukin-4 (IL-4), IL-10, and IL- 12). Nonetheless, perpetuation of the host response due to a persistent bacterial challenge disrupts homeostatic mechanisms of control and results in the release of mediators including pro-inflammatory cytokines (*e.g.*, IL-1, IL-6, tumor necrosis factor-α [TNF-α]), proteases (*e.g.*, matrix metalloproteinases), and prostanoids (*e.g.*, prostaglandin E2 [PGE2]) which can promote extracellular matrix destruction in the gingiva, periodontal ligament and stimulate bone resorption [12].

Clinically, periodontitis results in the formation of soft tissue pockets or deepened crevices between the gingiva and tooth root. Loss of the periodontal ligament and disruption of its attachment to the cementum, as well as resorption of alveolar bone, also occur. Together with loss of attachment, it attains an apical migration of the epithelial attachment along the root surface and the resorption of neighboring bone [13,14].

Treatment of periodontitis on a first base is directed to the control of the cause (causal therapy), *i.e.*, reduction of the bacterial load on the tooth surface and sulcus, by mechanical treatment above and below the gum level (scaling and root

planning, respectively), pharmacologic treatment (anti-inflammatory and antibacterial local and/or systemic approaches) when indicated, and instructing and motivating the patient for reinforced home oral hygiene techniques [15, 16]. These conventional nonsurgical therapeutic approaches and surgical periodontal flap procedures are generally successful in halting the progression of the disease but broadly result in soft tissue recession - associated with poor aesthetics and result in residual pockets formation. Further, which are thus difficult to clean effectively and lessen (affect) long-term prognosis. These limited outcomes can be circumvented or underrated by periodontal regenerative procedures that aim to restore the lost function and anatomic organization of the periodontal structures.

1.2. Bone grafts in Periodontal Regenerative Approaches

The goal of periodontal regeneration is the restoration of the periodontium to its original anatomical form and biological function. Regenerative periodontal therapy aims to predictably restore the tooth's supporting periodontal tissues and should result in formation of a new connective tissue attachment (*i.e.* new cementum with inserting periodontal ligament fibers) and restoration of the lost alveolar bone level [17]. Clinical outcome parameters consistent with successful regenerative therapy include - reduced probing depth, increased clinical attachment level and radiographic evidence of bone fill.

Bone replacement grafts, including autogenous grafts from intraoral or extraoral sites, allografts, xenografts, and alloplastic bone substitutes are the most widely used treatment modalities for the regeneration of periodontal osseous defects. They seem to provide a structural framework for clot development, maturation, and remodeling in addition to initiating the biological processes that support bone formation in the established osseous defects. Bone grafting materials also exhibit a variable capacity to promote the coordinated formation of bone, cementum, and periodontal ligament when placed and retained in periodontal defects [18].

Bone grafts can play an important role in the correction of the osseous aspects of periodontal defects, either by the process of osteogenesis, osteoinduction or by osteoconduction [19]. An osteogenic material, such as cancellous bone/bone marrow, contains living cells that are capable of differentiation and formation of new bone tissue. Alternatively, osteoinductive materials can induce bone

formation by recruiting undifferentiated mesenchymal cells and guide them into the osteogenic pathway, whereas osteoconductive materials act principally as an inert scaffolding support for new bone formation [19,20].

Various systems have been used to classify bone replacement grafts. Generally, these are sorted according to its source, chemical composition and/or physical properties. However, current advances in material and biological sciences have increasingly blurred the existing boundaries between classes. Here, we focus on a classification system based on the origin of the graft, categorizing them into autografts, allografts, xenografts and alloplastic materials. Historically, autografts were the first bone replacement grafts to be reported for periodontal applications, while allogenic freeze-dried bone was introduced to periodontics in the early 1970's. Demineralized allogenic freeze-dried bone gained wider application in the late 1980's while xenografts and alloplastic materials application for periodontal use occurred around the same time [21].

Autografts

For many years, autografts have been considered the gold standard for bone regenerative clinical applications. The decision to use autogenous grafts necessitates consideration of the donor site, procurement technique and handling or processing of the harvested material. Autogenous bone can be harvested intraorally (*e.g.*, from the chin area, tuber region, healing extraction sites and toothless jaw segments), with or without processing, to yield graft materials of different forms (*e.g.*, cortical chips, osseous coagulum and bone blend) [22]. Alternatively, extraoral sites (*e.g.*, iliac crests, ribs, cranium and tibial metaphyses) can be used [23]. Autogenous grafts are nonimmunogenic and contain osteoblasts and osteoprogenitor stem cells, which are, theoretically, capable of proliferating and differentiating into the osteogenic lineage.

Autogenous bone grafts have been applied to periodontal defects. Histologic findings from early case reports, as well as subsequent radiographic and clinical data attained from large case series and controlled clinical trials substantiate the potential use of autogenous bone/bone marrow grafts (from either introal and extraoral origins) to support periodontal regeneration in humans [19,24]. Nonetheless, one should consider that there are limitations to obtaining

autogenous grafts, such as insufficient oral sites for harvesting, the requirement for a second surgical site and expected morbidity at the donor site. Moreover, autograft harvesting is associated with an estimated 8.5–20% of complications, mostly including the possibility of hematoma formation, blood loss, nerve injury, hernia formation, infection, arterial injury, fracture, cosmetic defects, tumor transplantation, and sometimes chronic pain at the donor site [22, 25,26].

Allografts

Allograft materials (*i.e.*, bone graft tissues transplanted between different individuals from the same species) have been used with success in the regeneration of the bone tissue. These allografts include frozen grafts, freeze-dried bone allografts and demineralized freeze-dried bone allografts [18]. The possibility of disease transfer, antigenicity and the need for extensive cross-matching has precluded the use of fresh frozen bone in current clinical applications. Additionally, the evidence that freeze-drying markedly reduces the antigenicity and other health risks associated with fresh frozen bone, as well as the favorable results obtained in the field trials with freeze-dried bone allografts have led to the extensive use of these grafts in the treatment of periodontal osseous defects [27]. The use of cancellous bone, rather than cortical bone is broadly recommended since cancellous allografts were proven to be less antigenic and also due to the increased proportion of bone matrix, and therefore the presence of more osteoinductive components in the cortical bone [28].

Further, research outputted that the bone matrix was responsible for blocking the biological availability of existing stimulating factors and thus, demineralized allografts were developed. Demineralized allogenic bone exhibits the capacity to induce bone formation in nonorthotopic sites, and is thus considered to be osteoinductive in nature. Bone demineralized to levels up to 2% residual calcium has been shown to provide maximum osteoinductive potential [29], presumably due to exposure of bone morphogenetic proteins [30]. Several works provided conclusive histologic evidence that demineralized allografts support periodontal regeneration in humans [18, 31].

The main advantages of using allografts are associated with the wide availability of the material and the absence of any donor site within the patient. Main reported

disadvantages include the process of preparing the graft (*i.e.*, freeze-drying and/or irradiating), which thus decrease the material's integrity and osteogenic potential, and the eventual immunogenicity of the material. Further, a major concern with allografts is the potential for disease transfer, particularly those associated with viral and prion pathogens [18, 19, 27].

Xenografts

Bone xenografts are naturally derived deproteinized cancellous bone grafts from another species, such as bovine, porcine, equine and natural coral. Following the withdrawal of the organic component, the enduring inorganic structure provides an organized matrix for new tissue ingrowth, thus maintaining the physical dimensions of the defect throughout bone regeneration [32]. However, there are still considerations regarding the immunological tolerance of the grafted tissues, the residual infection risk and a limited patient acceptance.

Mammals-derived bone xenografts present porosity and mineral content comparable to that of the human bone and perform as osteoconductive scaffolds, guiding the new bone tissue formation. These xenografts are prepared by chemical or low-heat extraction of the organic component from animal's bone. Histological evidence of the bone regeneration in periodontal defects has been attained with the use of bovine xenografts [33].

The calcium carbonate exoskeleton of coral species can be processed into hydroxyapatite by hydrothermal exchange. The porosity and pore size distribution of the attained material, which is highly dependent to the species of origin, provides an osteoconductive scaffold for bone growth, undergoing timely dissolution and resorption through the bone remodeling process. Evidence of successful periodontal regeneration has been attained with the use of these xenografts [34].

Alloplastic Materials

New-generation alloplastic materials are biocompatible, inorganic-derived, synthetic bone substitutes. They possess some of the desired mechanical qualities of bone as well as osteoconductive properties, but are largely reliant on

neighboring viable periosteum/bone for the attainment of clinical success. They primarily function as defect fillers.

Alloplastic materials with relevance in periodontal applications can be classified as polymers and ceramics/glasses, the later including bioactive glasses, calcium phosphate cements, bioceramics and multiphasic materials.

Polymers

Polymers are potential candidates for periodontal bone grafting procedures and there is a wide range of available preparations with different physical, mechanical, and chemical properties. The polymers with clinical application can be loosely divided into natural polymers and synthetic polymers. These, in turn, can be sub-divided into degradable and nondegradable [35]. Natural polymers include polysaccharides (*e.g.*, agarose, alginate, hyaluronic acid, chitosan) and polypeptides (*e.g.*, collagen, gelatin). Regarding their successful clinical application, one may not overlook the limited structural properties of natural polymers (*i.e.*, a comparatively weak mechanical strength and variable rates of degradation) that restrict their application as standalone grafting biomaterials. Synthetic polymers (*e.g.*, poly (glycolic acid), poly (L-lactic acid), polyorthoester, polyanhydride), on the other hand, provide an easy manipulation of their properties, which can be effortlessly tailored into the desired characteristics of scaffolds for tissue engineering applications. In orthopedics, synthetic polymers have an extended use as injectable and solid products for bone tissue regeneration [36]. In periodontal applications, polymers have been more widely used as barrier materials, as part of guided tissue regeneration (GTR) applications, and their application for the regeneration of intrabony defects seems to be far more limited [37]. Recently, some clinical reports provide evidence for the effectiveness of a microporous polymer containing polymethylmethacrylate, polyhydroxylethylmethacrylate and calcium hydroxide, in the treatment of periodontal intraosseous defects [38,39].

Bioactive Glasses

Bioactive glasses are silicone-based, osteoconductive materials that bind to bone through the formation of carbonated hydroxyapatite. When exposed to body fluids, bioactive glasses are expected to be covered by a double layer composed of

silica gel and a calcium-phosphorous rich apatite layer. The latter promotes adsorption and concentration of proteins utilized by osteoblasts to form a mineralized extracellular matrix. It is believed that these bioactive properties guide and promote osteogenesis, allowing rapid formation of bone [40]. Periodontal clinical parameters assessment revealed an increase in the clinical attachment level and hard tissue fill, when intrabony defects were implanted with bioactive glass. Nonetheless, this biomaterial exhibits essentially osteoconductive properties and histologic analysis of human periodontal defects revealed that attained healing was based on connective tissue encapsulation of the graft material and epithelial down-growth – with minimal evidence of regenerated cementum or connective tissue attachment [41].

Calcium Phosphate Cements

In orthopaedic and bone tissue engineering applications, calcium phosphate cements are gaining special

interest due to their biomimetic nature and potential use as controlled release systems. Common components of the powder, besides tetracalcium phosphate, dicalcium phosphate dihydrate or anhydrous, include monocalcium phosphate monohydrate and anhydrous, octacalcium phosphate, tricalcium phosphate, hydroxyapatite and fluorapatite [42]. The liquid phase includes water, calcium- or phosphate-containing solutions, organic acids or aqueous solutions of polymers [42]. The primary role of the liquid is to provide a vehicle for the dissolution of the reactants and precipitation of the products. The fabrication of calcium phosphate cement is a versatile process which yields a variety of injectable pastes and set cement materials, with a wide range of physicochemical and mechanical properties [43]. These results are broadly dependent on the characteristics of the solid and the aqueous phase, as well as conditions in which the mixing reaction is conducted. One feature of particular interest in these cements is that they are intrinsically porous, with a relevant percentage of porosity within the nano to submicron range [43]. In periodontal applications, clinical implantation of calcium phosphate cements has reported controversial results, it has been shown to perform better than hydroxyapatite ceramic granules [44] and failed to demonstrate superior clinical outcome in comparision to open flap debridement [45]. A recent work showed that

at 6 months, intrabony defects implanted with calcium phosphate cements revealed a probing depth reduction and a gain the clinical attachment level. However, no site showed periodontal regeneration and there was no histological evidence of new bone formation. Moreover, the presence of new cementum and new organized connective tissue were residual [46].

Bioceramics

Bioceramic alloplasts have been the most used materials in periodontal regenerative approaches. They comprise mainly calcium phosphate materials, with a calcium/phosphorous relation similar to that of the human bone. The two most widely used forms are tricalcium phosphate and hydroxyapatite. These materials can be produced in either amorphous or crystalline phases, maintaining the same calcium and phosphorous ratios. Attained differences in the degree of crystalline arrangement are able to induced changes in physic-chemical and biologic characteristics of the developed ceramics.

Tricalcium phosphate includes both alpha- and beta- forms which are produced similarly, nonetheless presenting different resorption properties. The crystal structure of alpha tricalcium phosphate is monoclinic and consists of columns of cations while the beta tricalcium phosphate has a rhombohedral structure. Clinically, beta tricalcium phosphate is most commonly used, undergoing a relatively fast resorption within 6-18 months, following orthotopic implantion within the bone. Despite the promising results in orthopaedic applications [47], beta tricalcium phosphate has provided contradictory evidences in both animal and clinical trials of periodontal regeneration [48,49]. Studies reported improvements in clinical measures (including reduction of the probing depth and gain in the clinical attachment level) after the treatment of intrabony periodontal defects with beta tricalcium phosphate, thus only minimal regeneration occurred, being the major portion of healing attained with the formation of a long junctional epithelium, with limited new connective tissue attachment [49]. Despite the ability of this material to allow for bone deposition and ingrowth in orthotopic sites, it has been shown to become broadly fibro encapsulated when placed in periodontal intrabony defects, failing to stimulate new bone growth, and to be retained (with residual graft particles present) following long implantation times [50].

Synthetic hydroxyapatite, with the chemical composition of $Ca_{10}(PO_4)_6(OH)_2$, has found widespread and successful clinical utilization in the enhancement of the periodontal bone ingrowth. Its degradation can be controlled by the amount of porosity and degree of sintering, thus originating both absorbable and nonabsorble forms of hydroxyapatite [51]. Dense non-resorbable hydroxyapatite grafts are osteophilic, osteoconductive and act primarily as an inert biocompatible bone defect fillers. Resorbable forms, with a characteristic slow resorption rate, act as a mineral reservoir of calcium and phosphorous and, at the same time, as a scaffold for timely bone ingrowth. Hydroxyapatite presents remarkable biocompatibility with little inflammatory response when implanted within connective and bone tissues. Histological evidence of new bone formation around and in the neighborhood of grafted porous hydroxyapatite fragments, with the presence of osteocytes, osteoblasts and a normal peripheral connective tissue without inflammatory reaction have been reported in the literature [52]. Recently, advances in hydroxyapatite presentations have led to the development of substituted hydroxyapatites, with *e.g.*, magnesion, silicon, fluoride, that seem to report improved biomechanical and biological behavior in orthopaedic applications. [53]. Nonetheless, little evidence of application of these materials to periodontal regeneration has been validated.

Multiphasic Materials

More recently, there has been a growing interest in the development of multiphasic calcium phosphate ceramics as scaffolding materials for bone regenerative applications. They seem to be more effective in bone repair/ regeneration than pure phase materials (*e.g.*, hydroxyapatite, tricalcium phosphate) and have a controllable degradation rate, thus favoring the timely bone regeneration and remodeling processes [54]. Clinical application of different multiphasic materials revealed the efficiency for bone filling, performance for bone reconstruction and efficacy for bone ingrowth [55-57]. Bonelike® is a novel marketed multiphasic material, with proven clinical success in the bone-related regenerative applications [58].

Bonelike®

Bonelike® is prepared by a liquid-phase sintering route, in which hydroxyapatite is reinforced with a glass of the P_2O_5-CaO system [58]. During the sintering process the glass reacts with hydroxyapatite, forming beta tricalcium phosphate, which is following partially transformed into alpha tricalcium phosphate, at higher

temperatures. The relative proportions of the tricalcium phosphate phases in the final microstructure depend upon several experimental factors, including the glass content and composition [59]. Attained composites reveal an increased bioactivity and improved biomechanical behavior in comparison to hydroxyapatite, due to the reducing of the grain size and porosity during the liquid sintering process of the materials' preparation. The bioactivity of Bonelike® is determined by an optimal balance of the least soluble phase of hydroxyapatite and most soluble phase of tricalcium phosphates. Further, the incorporation of different ionic species, such as carbonate, magnesium, sodium and fluoride, result in the development of a material with a chemical composition most similar to the one of the mineral phase of the human bone. In terms of biomechanical properties, and comparing to hydroxyapatite, Bonelike® presents significantly higher values for flexural binding strength and fracture toughness [60].

Bonelike® grafts are currently being used in several successful clinical applications namely in orthopedic and oral/maxillofacial procedures. In orthopedic applications, it has been employed for the regeneration of several bone defects caused by trauma or ageing [61, 62]. Within the oral/maxillofacial area, Bonelike® has been used for the regeneration of maxillary and mandible bone, after cyst removal, impacted teeth extraction, for sinus lift and bone augmentation around implants, as well as for maxilla and mandible reconstruction [63-66]. Recently, it has been used with success in the regeneration of intrabony periodontal defects in a case-series of patients with aggressive periodontitis [67].

1.3. Clinical Management of Periodontal Defects

Successful bone graft therapy in periodontal application relies on the basic principles of periodontal surgical technique, including a thoughtful case/defect selection, cautious pre-operative and post-operative management, and a meticulously surgical technique. Some studies have investigated the possible sources of variability in the clinical success of bone grafting procedures in periodontal surgery and elected the following factors as determinant.

Patient Related Factors

Patient-related factors in the prognosis of the success of grafting periodontal regenerative interventions include the degree of plaque control, presence of residual periodontal infection, use of tobacco, drug administration, patient's

compliance and presence of systemic-associated condition, including diabetes, hyperparathyroidism, thyrotoxicosis, osteomalacia, osteoporosis and Paget's disease [17, 68].

Morphology of the Defect

The characteristic morphology of the intrabony defect seems to greatly determine the prognosis of the regenerative approach. Horizontal patterns of alveolar bone loss seem to be marginal responsive to periodontal regenerative therapies, including bone graft implantation; while vertical or angular bony defects, counting furcation defects, are often responsive to periodontal regeneration. Among the factors related to the anatomy of the defect, depth of the intrabony component and probing depth are consistently found to be relevant, as well as the number of residual bony walls defining the defects with two and three bony walls respond more favorably to treatment than do one-wall defects [17, 68].

Selection of the Graft Material

Selection of grafting material should be guided by the following principles: biologic acceptability, clinical predictability, degree and rate of resorbability, clinical feasibility, minimal operative hazards, postoperative sequelae and patient acceptance [17, 33, 68]. Also, only materials with a particle size range between 125 μm and 1000 μm should be employed. Particles with less than 100 μm in size elicit a pro-inflammatory response and are readily taken by macrophages, being rapidly resorbed with little or no new bone formation [69].

Surgical Procedure

The surgical technique for the treatment of periodontal intrabony defects with bone replacement grafts is essentially the same regardless of the type of graft material being used. Incisions are designed to allow for primary closure of flaps to protect the graft site from infection and the graft material from displacement [70]. Broadly, intrasulcular incisions are the common choice, with emphasis on preserving interdental tissue. Full thickness flaps are reflected to expose the underlying osseous defects and allow access for a thorough debridement of the defects and meticulous root planing [70]. Complete defect debridement and root

surface decontamination are essential before placement of the bone graft material. Accordingly, the periodontal defect should be debrided of all soft tissue using hand, ultrasonic, and/or rotary instruments. Moreover, the meticulous removal of all hard and soft accretions on the root surface and any clinically affected cemental surface and root abnormalities is mandatory. Following root debridement and preparation of the defect site, the bone grafting material should be carefully implanted, lightly condensed, and contoured to mimic the normal architecture of the adjacent alveolar bone. After suturing, slight pressure on the facial and lingual flaps should be applied to minimize the clot beneath the flap. Following, a periodontal dressing should be placed to protect the wound, without displacement of the graft or compromising the blood supply to the gingival flaps.

Selection of a specific flap design, in relation to anatomical characteristics of interdental space and location/morphology of bony lesion, and proper suturing technique may significantly contribute in determining the amount of soft and hard tissue changes following surgery [71]. This was confirmed by a significant center-related effect on treatment outcome observed when a biomaterial/ regenerative therapeutic intervention has been evaluated in a multicenter trial [72].

2. CLINICAL CASE

2.1. Case Presentation

A male patient with aggressive periodontitis and the presence of two intrabony periodontal defects of similar morphology was enrolled into a split-mouth design, in order to address the regenerative capabilities of an alloplastic material (Bonelike®, from Medmat Innovation). In this design, the test site comprised the implantation of Bonelike® following surgical debridement, while the control site was treated only with surgical debridement.

Clinical and radiological data were recorded at baseline and following 6 months post-operatively, for both control and test site, and are presented on Table **1**. Pre-operative radiographic (Fig. **1**) and tomographic images (Figs. **2-4**) allowed for the computation of radiological parameters. Clinical parameters were assessed with periodontal probing (Fig. **5**).

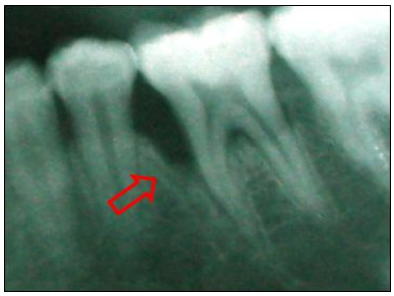

Figure 1: Preoperative radiographic image of the area around the mandibular left first molar, suggesting advanced bone loss at the mesial aspect (test site).

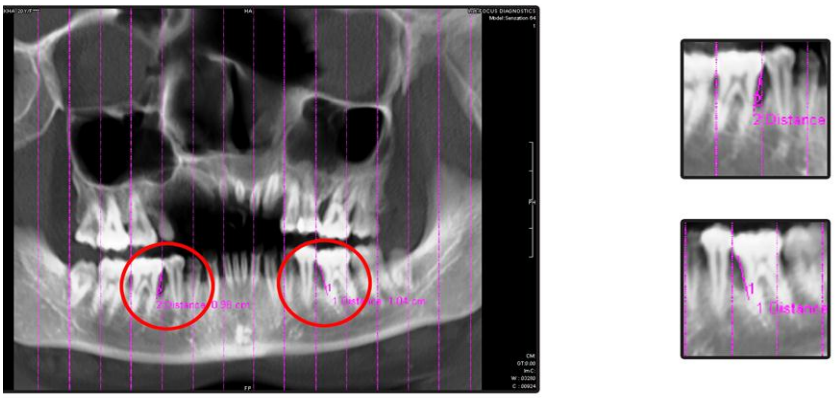

Figure 2: Tomographic image showing the linear measurements on panoramic view - from cement-enamel junction (CEJ) to base of defect (BOD). The area of interest in the panoramic view is encircled and shown in the inset. Copyright Quintessence Publishing Co Inc.

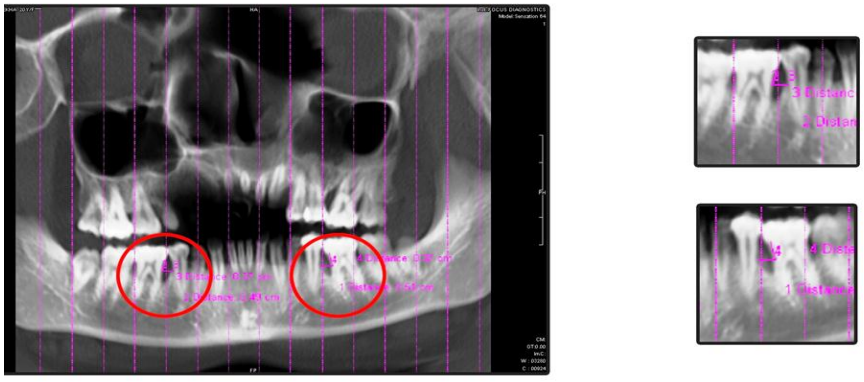

Figure 3: Tomographic image showing the linear measurements on panoramic view- from CEJ to alveolar crest (AC). The area of interest in the panoramic view is encircled and shown in the inset. Copyright Quintessence Publishing Co Inc.

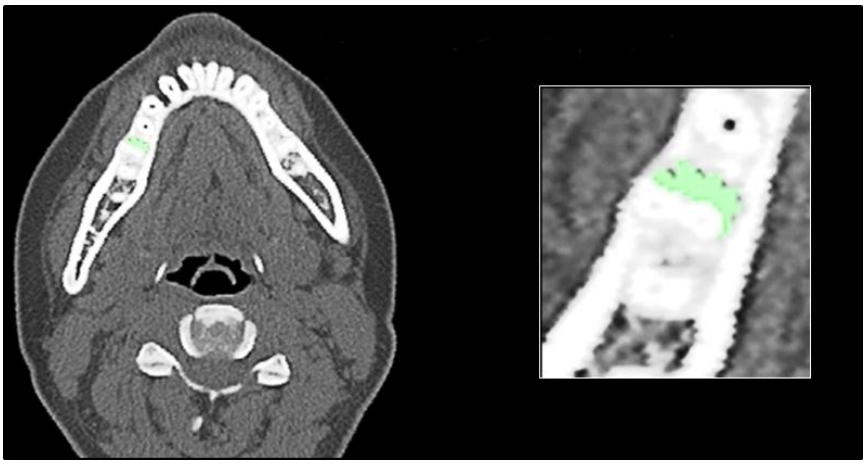

Figure 4: Mapping of the defects volume on axial section. The defect outline depicted in green color is shown in the inset. Copyright Quintessence Publishing Co Inc.

Figure 5: Preoperative measure of the probing depth using an acrylic stent and a UNC 15 periodontal probe.

2.2. Surgical Procedure

Routine periodontal pre-operative preparation was performed and local anesthesia was given. A crevicular incision was given which allowed for flap elevation, by means of blunt dissection with the help of a periosteal elevator. All the granulation tissue was carefully removed to ensure a clean site, and this was followed by thorough root planing. Further, during surgical protocol, utmost care was taken to preserve the interdental papilla (which aims to allow a better interproximal coverage of the graft material and to prevent exposure and exfoliation of the graft). An intra-operative image following defect debridement is shown in Fig.**6**.

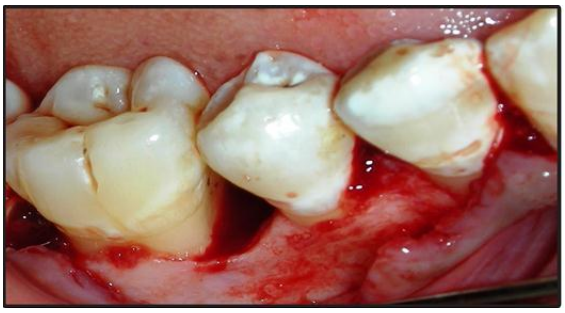

Figure 6: Operative view following the reflection of a full thickness flap (assessed by means of a sulcular incision) and defect debridement. The defect is visualized at the mesial aspect of the first molar.

Following, an adequate quantity of the graft material (Bonelike®) was dispensed into a sterile dappen dish and mixed with an adequate quantity of patient's own blood drawn from the defect area. Before the graft implantation, a 3-0 non resorbable suture was passed through the buccal and lingual papillae and the suture was left loose (in order to prevent removal of the graft particles by the passage of the needle). At the test site, the alloplastic material was carried into the defect using a cumine scaler, in small increments, and filled to the level of the alveolar crest. The graft was then gently compacted and adapted to the anatomical contour of the alveolar bone. At the control site, the defect area was closed with sutures following surgical debridment alone. An intraoperative image of the test site is shown on Fig.**7**.

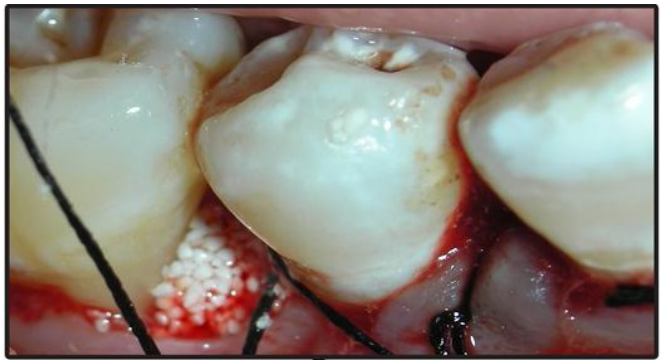

Figure 7: Intraoperative image of the allograft material contained within the defect.

The pre sutures placed were tightened and completed using routine interrupted interdental sutures, as shown in Fig. **8**. This allowed the repositioning and securement of the mucoperiosteal flaps.

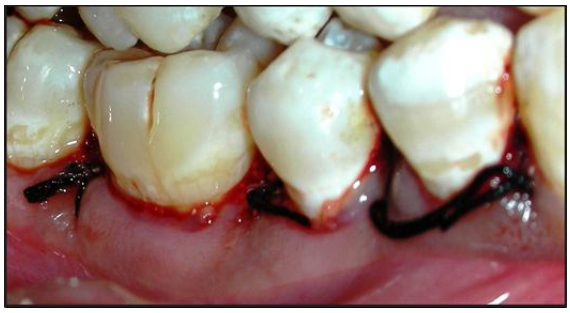

Figure 8: Intraoperative view of the sutured flap showing a complete coverage of the grafted location.

A non-eugenol based periodontal dressing (Coe Pak®, from GC Europe) was immediately placed at the surgical site, as shown in Fig. **9**.

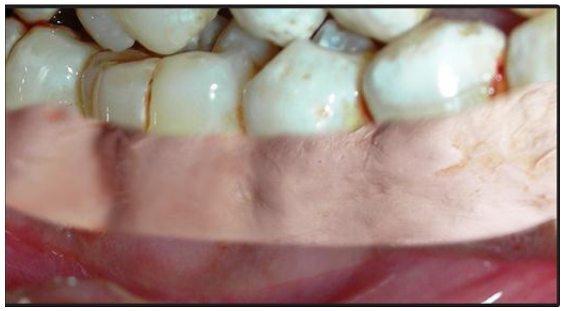

Figure 9: Intraoperative view of the placed periodontal dressing.

Routine postoperative instructions were carefully given to the patient and antibiotics and analgesics were prescribed, throughout the first 7 days following surgery. The patient was recalled 7 days after the surgical intervention and the periodontal dressings and sutures were removed. The patient was instructed to gently brush the area with a soft-bristled toothbrush. Patient was continuously monitored at 1, 3 and 6 months and, at each visit, oral hygiene was assessed and oral hygiene instructions were reinforced. At 6 months, all soft tissue measurements were repeated and a CT scan was taken to address hard tissue outcomes.

2.3. Results and Discussion

This clinical case aimed to address the response of periodontal intraosseous defects, treated by open flap debridement with and without the implantation of a glass

reinforced hydroxyapatite alloplast (Bonelike®). The grafted material was well tolerated without any clinical signs of inflammation, infection or impaired healing.

Attained results revealed that both treatment modalities (surgical debridement, at the control site and bone graft implantation, at the test site) resulted in the reduction of the probing depth and in the gain of the clinical attachment level - the assessed clinical parameters associated with improved periodontal health. The reduction in the probing depth, from baseline till 6 months post-operatively was higher in the test group, comparing to control (4 mm *versus* 2 mm, respectively), as well as the gain in the clinical attachment level (4 mm *versus* 2 mm, respectively).

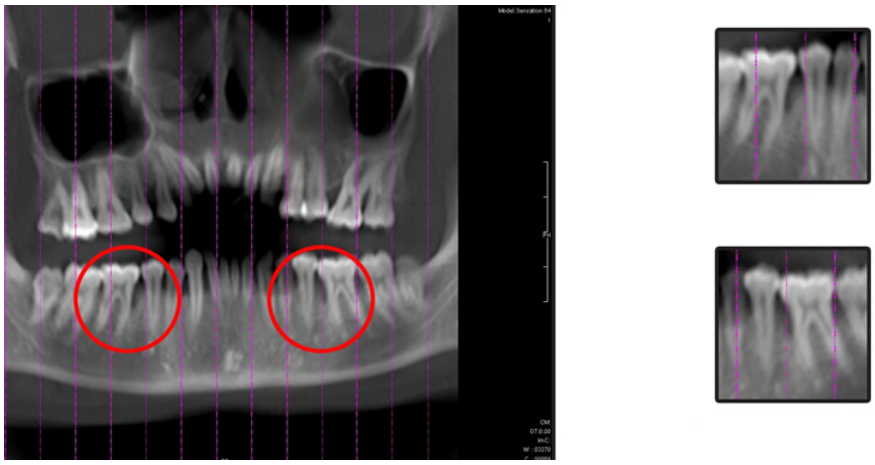

Figure 10: Tomographic image showing the regenerated area both in the control and test sites. Areas of interest in the panoramic view are encircled and shown in the inset. Copyright Quintessence Publishing Co Inc.

Conducted tomographic analysis (Fig. **10**) also revealed a reduction in bone defect depth, from baseline to the final evaluated time point (6 month postoperatively), both at control and test sites (1.5 mm and 2.4 mm, respectively) and in the defect volume, which reduced 35.9 mm^3 in the control site and 56.3 mm^3 in the test site. Results are summarized in the Table **1**. Percentage of bone defect fill was following calculated according to the following equation,

$$\frac{(Baseline\ bone\ volume\ -\ Final\ bone\ volume)}{Baseline\ bone\ volume} \times 100 \quad \textbf{(1)}$$

and revealed a result of 58.56% for the control site and 68.57% for the test site.

Table 1: Clinical and radiological parameters at baseline (preoperatively) and 6 months postoperatively for the control and test sites.

Parameter	Control site (baseline)	Control Site (6 months post-op.)	Test Site (baseline)	Test Site (6 months post-op.)
Probing depth (mm)	7	5	8	4
Clinical attachment level (mm)	8	6	9	5
Bone defect depth (mm)	4.5	3.0	5.2	2.8
Defect volume (mm^3)	61.3	25.4	82.1	25.8

Bone defect depth was calculated from the difference between CEJ – BOD measure and CEJ – AC measure, at the tomographic analysis, as shown in Figs. 2 and 3.

These results demonstrated that the site treated with Bonelike® implantation showed an increased bone defect fill, comparing to the surgical debridement alone. Tomographic and clinical measures substantiate the attained findings. Bonelike® seems to adequately induce new bone tissue formation in intrabony periodontal defects, in a greater extent than surgical debridement alone. These results are in accordance with those published by Kumar *et al.* that showed the successful application of Bonelike® in the regeneration of periodontal bone defects [67,73].

Several literature reports aimed to address the impact of periodontal bone graft procedures in the treatment/regeneration of periodontal intraosseous defects. Recently, two high evidence literature reports, meta-analysis assessed systematic reviews, have been published and aimed to address the proposed questions.

Trombelli *et al.* aimed to determine the adjunctive effect of grafting biomaterials with open flap debridement in the treatment of deep intraosseous defects [74]. In this systematic review, the authors addressed a wide range of studies implanting several bone grafting materials into periodontal intrabony defects. These included autologous bone grafts, bone allografts, coralline xenografts, bioactive glass, several formulations of hydroxyapatite of both synthetic and biological origin, in either porous and dense forms, polylactic acid and a polymethyl methacrylate/

polyhydroxyl-ethyl methacrylate composite. Apart from the implantation of polylactic acid, the implantation of all the other bone substitutes produced favorable changes in clinical attachment level and probing pocket depth – clinical measures of periodontal health - and increased defect fill when compared with open flap debridement alone [74]. Nonetheless, the authors verified a marked heterogeneity in the attained results from different studies, in each group of the assessed biomaterials.

The systematic review of Reynolds *et al.* aimed to address the efficacy of bone replacement grafts in proving demonstrable clinical improvements in periodontal osseous defects compared to surgical debridement alone [19]. The authors showed that bone graft implantation allowed for an increase in the bone level and in the clinical attachment level, and a reduction in the crestal bone loss and in the probing pocket depth, as compared to surgical debridement alone [19]. Further, they showed no significant differences in the clinical measurements between the use of synthetic hydroxyapatite and bone allografts and concluded that bone replacement grafts provide demonstrable clinical improvement of the periodontal bone regeneration, compared to surgical debridement alone [19].

3. CONCLUSIONS

There is substantial clinical and histological evidence that support the concept that autogenous bone grafts and demineralized freeze-dried bone allografts are effective regenerative materials in the treatment of periodontal intrabony defects. Moreover, several synthetic alloplastic materials have also been proven to be of clinical utility, enhancing the process of periodontal regeneration. In this work, Bonelike®, a glass-reinforced hydroxyapatite was implanted in an intrabony periodontal defect and has demonstrated an increased regeneration (assessed by clinical and radiological parameters), in comparison to surgical debridement alone. Despite the need for additional controlled clinical trials, preliminary data substantiates the applicability and prospective successful clinical application of Bonelike® in periodontal regenerative applications.

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Declared none.

CONFLICT OF INTEREST

The contents presented in the chapter have been carefully written based on the review from the references cited and the results obtained from the investigations carried out by the authors. Further, there is no conflict of interest with other people or organizations in respect of the present research work. Also, there is no financial support from any other organizations.

DISCLAIMER

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