'Biomaterials for bone Regenerative Medicine'

Transtech Publishers, Switzerland

CHAPTER 6

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CHAPTER 6

Calcium Phosphate-based Materials for Bone Regenerative Medicine

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1. INTRODUCTION

Regenerative medicine uses graft procedures aiming at repairing or replacing any defective, diseased tissues or organs by trauma, ageing, etc. The use of bone graft is required to restore skeletal integrity and enhance bone healing in several orthopaedic, dental and maxillofacial procedures. In fact, bone is just second to blood as a material most often implanted in the body. There are, annually, over 500 000 to 600 000 bone graft procedures in the United States and 2.2 million worldwide [1, 2].

There are several types of bone grafts, namely, autograft, allografts, and xenografts, however all of them have advantages and disadvantages. Autografts are considered the gold standard graft due to the lack of immunological response and the ability to provide intrinsic osteoinductive growth factors, osteogenic cells and structural scaffolds [3]. The use of autograft, whilst often effective has several disadvantages, such as additional invasive surgical harvest procedure, increased blood loss, limited graft amount and extra morbidity to the patient [4, 5]. As an alternative, allografts can be used. Allografting procedures are less successful then autografts. The processing of allograft tissue does not completely eliminate the risk of transferring viral agents, such as HIV, hepatitis B virus and hepatitis C virus [6]. Furthermore, the numerous allograft preparation, preservation and sterilization methods may modify their physical-chemical properties and concomitantly influence biological and mechanical behaviour of the graft, therefore dictating the success of allogenic bone transplantation [6]. Another determinant factor is the immunological reaction that allografts may generate in the organism [7]. When bone from one species is implanted into a member of different species is designated by xenografts. These grafts are usually bovine in origin and are submitted to deproteinization processes in order to eliminate immunogenicity and putative infectious risk. Actually, these processes reduce but do not completely eliminate the risk of disease transmission and immunological response [7].

Therefore, the limitations in autografts and allografts have led to great advances in the development of synthetic alternatives. Among them, synthetic materials, such as calcium phosphate ceramics (e.g. hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$, HAp), tricalcium phosphate ($Ca_3(PO_4)_2$, TCP), dicalcium phosphate ($Ca_2P_2O_7$, DCP) and tetracalcium phosphate ($Ca_4P_2O_9$, TeCP), etc.) are promising graft materials used in the bone tissue regenerative process [8].

Synthetic bone grafts, such as calcium phosphate-based materials, display similar chemical and biological properties to human bone and offer many advantages compared to autografts. These graft materials do not possess the risk of disease transmission and eliminate the need for an additional surgical procedure for transplantation, reducing patient pain and recovery time. Hence, calcium phosphate-based materials are gaining growing interest for use as bone synthetic graft materials [3, 6, 8].

Successful bone grafting, has to follow four basic criteria, namely, osteoinduction, osteoconduction, osteogenesis, and stability [9]. Osteoinduction refers to the recruitment, proliferation and differentiation of osteoprogenitor cells into osteoblasts which are responsible for the new bone formation. Osteoconduction is the process, which provides a structural framework and environment that supports the migration, attachment and growth of osteoblasts and osteoprogenitor cells into the graft. Osteogenesis refers to cellular process of new bone formation by osteoblasts following osteoinduction. And finally, stability, or movement resistance ability at the union site, is crucial to avoid delayed neovascularisation, which could result in an inadequate growth of the newly formed bone over the bone graft leading to pseudoarthrosis. Usually, synthetic bone grafts do not gather all four criteria; however they display several degrees of osteoconductivity and materials, such as bioactive glasses, silicon-substituted apatite's , glass reinforced HAp and synthetic calcium phosphate ceramics are of special interest for bone repair or regeneration [3, 6, 8, 9].

2. BIOACTIVE GLASSES AND GLASS-CERAMICS

Bioactive glasses are amorphous materials and glasses ceramics are polycrystalline materials composed of one or more glassy and crystalline phases. They are usually obtained from a base glass, most commonly produced either by the sol gel or the more conventional technique of melting, which is next followed by a heat treatment, at an adequate temperature cycle, to be converted into a glass or glass-ceramic material. Bioactive glasses are amorphous and glass-ceramics are obtained by devitrification of glass, although the vitreous phase prevails on the crystalline phases. Thus, the control of heat treatment cycle in the preparation of glass ceramics is of extreme importance in order to achieve a final microstructure with desirable properties [10-13].

Bioactive glasses are typically prepared from high-purity raw materials as their quality strongly influences that of the end-product. Almost all of the bioactive glasses and glass-ceramics currently used contain large amounts of silica (SiO_2). Nevertheless, the silica-free calcium phosphate glass-based materials also have high potential for being used as bone graft substitutes [14, 15]. Additionally, specific additives may be incorporated into the base glass composition to induce the nucleation and growth of particular crystal phases, within the residual vitreous matrix, with specific physical-chemical properties in order to obtain glass-ceramics. More specifically, it allows the preparation of glass-ceramic materials with the presence of biocompatible and bioresorbable phases. The characteristics of the final constituent phases and microstructure of the glass-ceramic establish its properties and main applications. There are three main techniques used for the preparation of bioactive glass and glass-ceramic materials; (i) casting and controlled crystallization, (ii) sintering and crystallization of glass powder, and (iii) sol-gel technique [12, 16].

Two of the most well known glass-based materials are Bioglass®, Ceravital® and Cerabone® A/W [17-20]. Several clinical applications of bioactive glasses and glass-ceramics are reported in the literature. Bioglass® was reported to be used in implants in middle ear surgery, alveolar ridge for denture wearers and in periodontal repair and maxillofacial reconstruction [21-24]. The Cerabone A/W glass-ceramic is reported to be used in replacing the iliac crest and in vertebral surgery [25-28].

When implanted in living tissues, all materials elicit a response from the host and generally both tissue and material undergo physical and/or chemical modifications. Based on these modifications, ceramics can be classified as; (i) nearly inert ceramics, (ii) surface reactive ceramics (bioactive) and (iii) bioresorbable ceramics [29-33].

The nearly inert ceramics such as alumina and carbons are chemically stable and elicit minimal response within the surrounding tissue, maintaining its characteristics throughout the entire period of implantation in the organism [34-36].

The surface reactive ceramics are midway between nearly inert and resorbable in behaviour. This kind of ceramic elicits a biological response to facilitate a direct chemical bond between the material surface and the surrounding tissues. The glass-based materials are considered surface reactive ceramics and some examples included in this group are bioactive glasses (Bioglass®) and glass-ceramics (Ceravital®, Cerabone A/W) [17, 21, 34, 37, 38]. When implanted, the materials undergo dissolution and release ions into the surrounding environment with consequent local pH changes. The composition of the materials controls their surface reactivity. These kinds of materials do not become encapsulated when implanted, but closely adhere to the surrounding living bone tissues [39, 40].

The glass and glass-ceramics that show the ability to bond to bone after implantation became known as bioactive ceramics.

Bioresorbable ceramics are designed to degrade progressively with time and be replaced with natural host tissue, without toxicity and rejection. This biodegradation may be caused by three factors; (i) physicochemical dissolution, which depends on the solubility of the material and the local pH, (ii) physical disintegration into small particles as a result of preferential chemical attack of grain boundaries, and (iii) biological factors, such as phagocytosis.

Bioresorbable materials may show some complications in the clinical use, such as (i) maintenance of strength and stability of the interface during biodegradation and replacement by the natural host tissue, and (ii) matching resorption rates to the repair rates of body tissues. This point is very important, since some materials display precocious resorption and some delayed resorption. Since a great concentration of ions or/and particles of a bioresorbable material is released, it is important that it consists only of metabolically tolerable substances, which restricts the material's compositional

design and therefore the mechanical behaviour and eventually its final applications [32, 36, 41, 42].

3. SILICON-SUBSTITUTED APATITES

Several theories have been developed regarding the beneficial effect of silicon in several metabolic processes including bone formation; so it is worthily to dedicate a section to this subject.

Silicon is one of the most common elements on earth. There are reports that in 1878 Louis Pasteur mentioned that "Effects of silicic acid are destined to play a great and major role in therapy"[43].

In the 1970`s, Schwarz and Carlisle presented very similar results [43-46] regarding the effect of silicon on bone development. Schwarz demonstrated that rats submitted to a deficient diet in silicon, not only had a slower growth, but an evident disturbance on bone development displaying shorter skulls and altered bone architecture. Schwarz also described an enzyme capable to remove silicic acid from a synthetic form, *silicase* [43]. This enzyme is membrane-bound located at the mitochondria and microsomes in the pancreas, stomach and kidney (lower concentration), it is a very stable enzyme [43]. Schwarz also proposed a correlation between the effect of silicon, arthritis, bone diseases (osteomalacia and osteogenesis imperfecta), scleroderma, wound healing and atheroscleorisis [43]. Carlisle presented similar results when chicks were subject to a diet poor in silicon, the work demonstrated the importance of silicon in bone growth and development [43, 47, 48]. Furthermore, Carlisle showed that silicon´s primary effect in bone and cartilage is related to cellular matrix formation, especially on bone mineralization. Silicon has also an important metabolic role in connective tissue structure, taking in consideration that silicon is present in high concentrations in metabolically active osteogenic cells [48]. According to the author a diet deficient in silicon is incompatible with the normal growth and skeletal development in these animals, they presented atrophied organs, retard skeletal development, less flexible legs, smaller skulls, flatter bones, articular cartilage with lower amount of cartilage, glycosaminoglycans and collagen, although most of this alterations can be correct by the addition of silicon to the diet [46].

Using an electron microprobe Carlisle demonstrated that silicon is present in active growth areas in young bone of mice and rats, the author also found a direct relation between the concentration of silicon and the degree of calcification, being the highest level detected at early stages of calcification [45]. Further studies, showed that osteogenic cells have larger amounts of calcium, phosphorous, magnesium and silicon, being the later on the most abundant anion on these cells. The increase on calcium content in bone undergoing the mineralization process is followed by a decrease in

silicon concentration, especially when the concentration of calcium reaches the values observed in bone apatite. The maximum amount of silicon observed was with calcium-to-phosphate (Ca/P) molar ratio of 0.7 (early stage of calcification) and decreases dramatically when Ca/P ratio reaches 1.67 (mature bone) [47]. It has been suggested that one of the most important factors in calcification is the binding of calcium; these studies suggested that silicon can play an important role in this process.

In 1992 Keeting *et al.*, [48] showed that Zeolite A, material that releases silicic acid and aluminium salts, stimulates proliferation and differentiation of osteoblast-like cells in culture and more recently Reffit *et al.,* [49] showed that at a physiologic concentration (10-20 μM) silicon stimulates collagen type I synthesis, alkaline phosphatase activity and osteocalcin synthesis in human osteoblast-like cells.

Taking in consideration the previous studies several researchers have focused their attention to the development and characterization of synthetic biomaterials containing silicon, namely silicon-substituted hydroxyapatite (Si-HAp), silicon stabilized tricalcium phosphate (Si-TCP) and several bioglasses containing silicon. *In vivo* studies performed by Patel *et al.* showed an enhanced bone apposition/ingrowth when comparing with stoichiometric Hap [50]. Botelho *et al.* demonstrated that the incorporation of silicon into HAp lattice induces a faster formation of an apatite layer, which induces the formation of a bond between the implanted material and the bone tissue. Additionally, at a protein level it was shown that the presence of silicon enhances the adhesion of human serum proteins in particularly collagen [51].

At a cellular level Botelho *et al.,* [52, 53] and Pietak *et al.,* [54] showed that the incorporation of silicon into an apatite enhances osteogenesis and osteoclastogenesis, two mechanisms essential for bone remodelling.

All these studies demonstrate that importance of silicon in several metabolic processes, most especially in bone development and calcification.

4. CALCIUM PHOSPHATE-BASED MATERIALS

Calcium phosphate-based materials are preferred as synthetic bone grafts in regenerative medicine applications. In fact, as main inorganic constituents of hard tissues in the body, calcium phosphate materials act as enhancers of bone restoration. They are generally used in dense, granular or porous forms, as well as coatings of metal prostheses and implants or in composite form [55, 56].

One of the most biocompatible material used today is HAp, $Ca_{10}(PO_4)_6(OH)_2$, and has been used as bone graft for a long time. HAp implant materials are osteoconductive, however they present slow resorption rates, remaining present in the organism for long periods of time [57].

Tricalcium phosphate exists in two allotropic forms as α-TCP and β-TCP and has been proved to be resorbable *in vivo*. The transformation of β-TCP to α-TCP takes place at around 1200 °C [58, 59]. However, due to a prompt resorption rate the use of α-TCP in biomedical field is limited [60]. On the other hand, β-TCP is a slowly degrading bioresorbable material and is regarded as an ideal material for bone substitutes since it suffers degradation concomitant with bone ingrowth [61-67].

Moreover, biphasic calcium phosphate ceramics were developed to better control the process of biomaterials resorption and bone substitution. Optimum balance of the more stable phase of HAp and more soluble TCP, would enable the gradual control of the dissolution in the body, seeding new bone formation as it would release calcium and phosphate ions into the biological medium. Some authors have defended the superior properties of the biphasic calcium phosphate materials "directly" prepared over those obtained by mixing two single phases [68]. The most commonly synthesis techniques used are precipitation [69-72] and the blending of different calcium phosphates [73]. Other techniques also employed are: solid state [74], treatment of natural bone [75], spray pyrolysis [76], microwave [77], and combustion [78], etc.

Santos *et al.,* have developed a glass-reinforced HAp material patented, registered and marketed as Bonelike®, an osteoconductive synthetic graft which is manufactured using a simple liquid phase sintering processof HAp in the presence of a vitreous phase [79]. Its composition has the advantage of mimicking the mineral composition of natural bone. In fact, the addition of $CaO\text{-}P_2O_5$ based glass into the HAp structure leads to the formation of secondary phases, α- and β-TCP. Their percentage is dependent upon the sintering treatment, content and the composition of the glass added. Due to the presence of α- and β-TCP in the HAp matrix of Bonelike® the mechanical properties of the material are improved. Furthermore, this biodegradable and bioresorbable phases allow a local enrichment in calcium, phosphorous and several ionic species, such as magnesium, sodium and fluoride, that in physiological conditions uphold a positive effect in the biomaterial's behaviour since they promote osteointegration and enhance bone regeneration [79]. Several Bonelike® medical applications are presented bellow.

5. BONELIKE® MEDICAL APPLICATIONS

Successful application of Bonelike® graft in several areas of bone regenerative medicine, namely in oral and maxillofacial surgery and orthopaedics, has been accomplished [80-88]. Bonelike® has been effectively applied in oral and maxillofacial surgery for bone augmentation around dental implants [80, 81] and for maxilary sinus floor elevation (sinus lift) [88] and in orthopaedics for the correction of valgus knee via open wedge high tibial osteotomies (HTO) [83].

5.1 ORAL AND MAXILLOFACIAL SURGERY

The evaluation, during implantology surgery procedures, of the direct bone bonding and osteointegration of the commercial pure Titanium (cp Ti) implants coated by plasma spraying with Bonelike® synthetic bone graft was performed. Bonelike®-coated retrieved implant showed excellent bone remnants on its surface without any tissue inflammatory signs. The reported Bonelike®-coated cp Ti implants improved primary stability, which may increase the lifetime of the implant [80, 81].

The application of Bonelike® during maxillary sinus floor elevation intended to overcome one of the major pre-surgical complications during this type of procedure, the rupture of the maxillary sinus membrane and consequent destabilization of the graft resulting in procedure failure. Currently, membranes, preferably resorbable, are used in order to occlude and secure the bone graft. The presented report, which included a 9 month follow-up, demonstrated that Bonelike® stabilized with calcium sulphate prevented the use of membranes and presented high predicable positive results in sinus lift surgeries [88].

5.1.1 IMPLANTOLOGY

The mostly used metals in implants and prostheses are titanium and cobalt-chromium alloys because of their strength, comparatively low stiffness, lightweight and bioinertness [89]. When metals are used as implantable materials their biocompatibility and osteointegration is lower when compared to bioceramic-coated metal implantable materials [90, 91]. The application of ceramic materials is usually made by the plasma-spray process in which heated particles are projected in a gas stream onto the implant or prosthesis [92]. Furthermore, calcium phosphate coated-implants, e.g. HAp coatings, have been employed in order to ensure faster fixation and firm implant-bone attachment due to extensive direct bone apposition. Nevertheless, reports that provided information regarding the interface bone-implant and on the fate of the coating over time, concluded that a success of a coating depends mostly on its chemical composition, thickness and crystallinity [93-96].

In vitro studies clearly demonstrated that Bonelike®-coatings present better characteristics for bone cell growth and function when compared with Hap- coatings ones [97-100]. Therefore, Bonelike® was employed as coating in order to promote osteointegration between cp Ti implants and host bone. Bonelike® coatings, unlike HAp coatings, display a higher degradation rate. This is due to the HAp liquid phase sintering in the presence of a vitreous phase that enables the formation of α- and ß-TCP. Therefore, controlled biodegradability of the material is possible, thus allowing early biological fixation [101]. Scanning electron microscopy (SEM) revealed the surface morphology of the Bonelike®-coated cp Ti dental implant material (Figure 1).

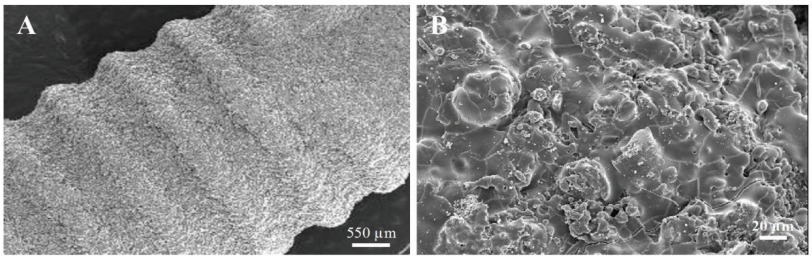

Figure 1: SEM image of Bonelike®-coated cp Ti dental implant material at magnification 10x (A) and 300x (B).

In the clinical study, 7 healthy citizens, 4 male and 3 female, ranging from 27 to 49 years with a mean age of 40 were considered. Twenty seven Bonelike®-coated dental implants were implanted in these 7 patients, 18 in the maxilla and 9 in the mandible, as shown in Table 1.

Table1: Clinical information of the 7 patients included in the clinical study and total number of Bonelike®-coated (cp Ti) dental implants that were used in the maxilla and mandible.

No.	Patient Information		No. Implants		Total No. of Implants
	Age	Sex	Maxilla	Mandible	
1	49	M	0	4	4
2	48	F	1	0	1
3	42	M	1	0	1
4	27	F	1	0	1
5	36	F	2	0	2
6	40	M	6	5	11
7	38	M	7	0	7

During the medical application of the Bonelike®-coated dental implants, patients were selected strictly on the basis of their clinical needs, according to the radiological and physical examination performed by their medical doctor [80, 81]. Including criteria were: any age; any sex; healthy citizens with no infected, non-characterized alveolar maxillar or mandibular lesions. Exclusion criteria were: systemic unhealthy subjects; infected cystic cavities; acute or chronic infection at local bone defect; bone

inflammatory diseases; osteomyelitis; malignant tumours; severe renal dysfunction; and patients with non-controlled bone metabolism.

The technique used for the implantation was the standard ad modum Bränemark [102]. In the present study, case No. 6, a 40-year-old male totally edentulous except 1.8 incluse, received 11 cp Ti Bonelike®-coated dental implants, 6 on the maxilla and 5 on the mandible. After 3 months, the central implant on the mandible was removed due to bad positioning. The maximum torque force applied was 40 N cm (Figure 2). The structure of the coating and new bone/implant interface of retrieved implant was evaluated using SEM and histological analysis using light microscopy. *In vivo* microstructure observations of Bonelike®-coated retrieved implant showed excellent bone remnants on its surface without any tissue and inflammatory signs observed (Figures 3 and 4).

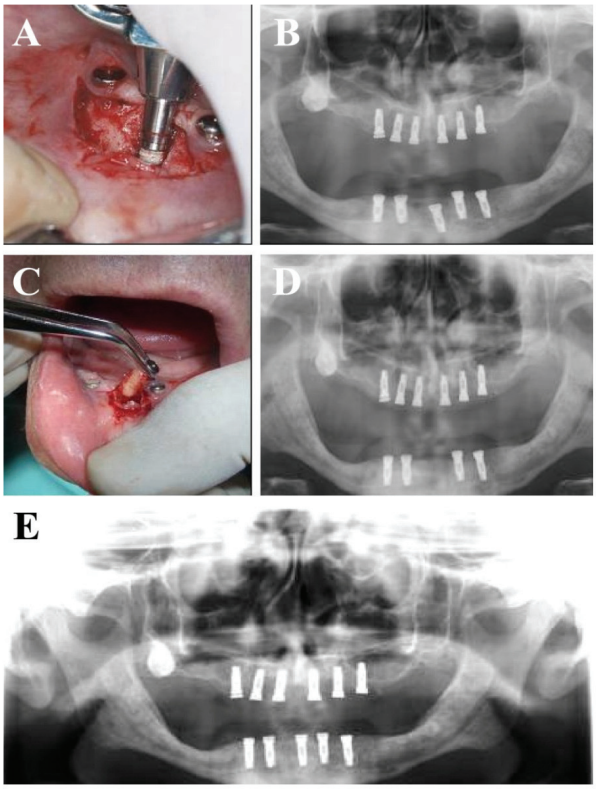

*Figure 2. Bonelike®-coated cp Ti dental implants clinical application. (**A**) An implantation of Bonelike®-coated dental implant in the mandible; (**B**) Postoperatory X-ray orthopantomogram; (**C**) After 3 months the central implant on the mandible was*

*removed due to bad positioning; (**D**) Postoperatory X-ray orthopantomogram after implant removal at 3 months postoperatory; (**E**) Postoperatory X-ray orthopantomogram of cp Ti Bonelike®-coated implants after a 6-month healing period.*

The histological analysis from the 3 month postoperatory biopsy sample is depicted in Figure 3. New bone ingrowth was observed surrounding Bonelike®-coated dental implants with a mature lamellar-like structure and a direct contact between the surfaces of the coating and the bone matrix established. No inflammatory cells and fibrous tissues were observed. Mature bone was clearly the major bone type observed at the time of retrieved sample (Figure 3A). New bone attached at cp Ti Bonelike®-coated implant bone interface was clearly observed in Figure 3B and 3C. Due to intimate bonding between new bone and Bonelike® it was almost impossible to distinguish any discontinuity at the interface (Figure 3B and 3C). Bio-affinity with highest osteointegration capacity and remodelling was clearly depicted in Figure 4C. Also radiological follow-up image (Figure 2E) showed a good osteointegration of the Bonelike®-coatings.

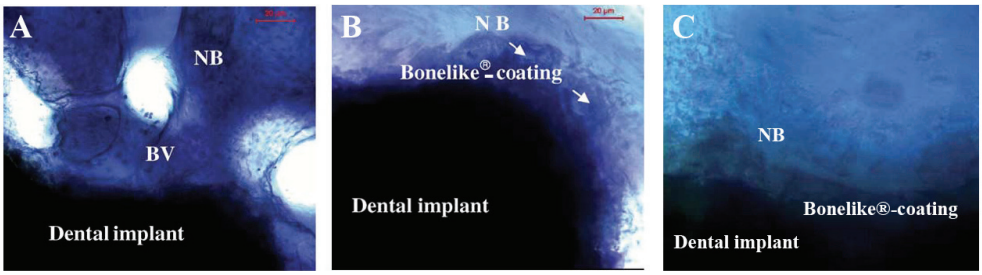

*Figure 3: Histological images (Solo-Chrome R staining) of the retrieved implant at 3 months postoperatory. Extensive bone regeneration and Bonelike®-coated implant/new bone interface at different magnifications: (**A**) 100×; (**B**) 200×; (**C**) 400×. (NB-new bone and BV-blood vessel).*

In a cross-sectional SEM view of the retrieved coated dental implant, it was evident the close adherence of the Bonelike® to the cp Ti dental implant substrate (Figure 4). At 3 months of healing period, besides the excellent attachment of the Bonelike® coating to the Ti-substrate (figures 4A and 4B), direct bone apposition to the coating was demonstrated (Figure. 4C and 4D). The new bone ingrowth was perceived through the micro and macroporosity of Bonelike® coating (Figures 4C and 4D). At the apposition interface of Bonelike®-coated cp Ti dental implant/new bone no gap formation was observed (Figures 4C and 4D). The new bone formed presented a high degree of maturation after 3-month implantation period (Figure4). The results of this report suggest that Bonelike® played a significant role in the new bone formation process

around the dental implants. Hence, Bonelike® proved to be an excellent coating for bone regeneration with outstanding behaviour for implantology applications [80, 81].

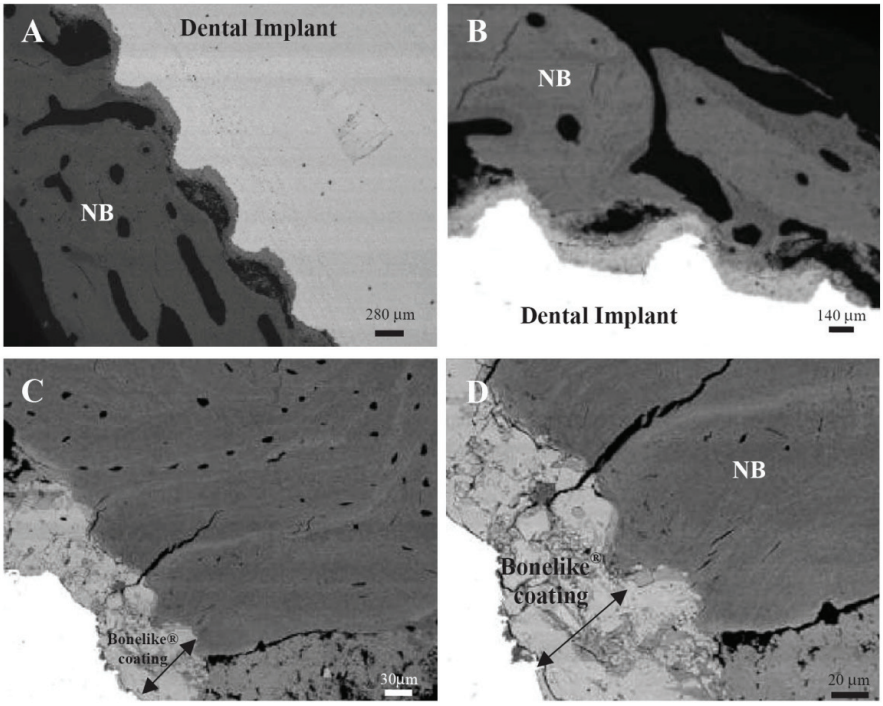

*Figure 4: SEM of the retrieved implant at 3 months postoperatory. (**A**, **B**) Extensive new bone formation and well adherent Bonelike® coating to the cp Ti-implant. Bone was directly apposed on the coating; (**C**) A thick coating well attached to substrate, demonstrating no significant Bonelike® coating dissolution after 3 months implantation; (**D**) New bone ingrowth through micro and macroporosity of Bonelike® coating, which remained attached to the substrate, without the formation of gaps at the interface. (NB-new bone).*

5.1.2 MAXILLARY SINUS FLOOR ELEVATION (SINUS LIFT)

The maxillary sinus floor elevation (sinus lift) is a used surgical procedure to obtain the placement of endoseous oral implants in the posterior portion of atrophic superior jaws, re-establishing a convenient osseous height and thickness to their insertion with adjusted length and diameter to receive and support occlusal forces [103-105]. Thus, through the opening of a lateral osseous window in the superior maxilla, technique described in the 1970s by Tatum [106], detachment and rising of the maxillary sinus membrane is obtained, creating a socket for the reception of the graft material.

As previously mentioned one of the more frequent pre-surgical complications of this procedure is the rupture of the maxillary sinus membrane, about 30 % of the times according to some reports, thus creating a communication between the receiving graft socket and maxillary sinus [107-110]. In some clinical cases, this complication has indication for an additional procedure, namely the resource to resorbable membranes in order to occlude the rupture and secure the granules.

Therefore, the current study aimed the use of Bonelike® under a stabilized formulation with calcium sulphate, in the ratio of 4:1. Such approach allowed avoiding practically the rupture of the maxillary sinus membrane, being only necessary the complete detachment and rising of the sinusal membrane [88]. The surgical procedure could be performed without granule loss or nasal sequestration. The respective follow-up, revealed the same advantages, since Bonelike® was steadily implanted with the graft stabilizer. Additionally, the use of Bonelike® graft alone was also possible and more attractive, since the resorption of calcium sulphate is circumvented and the osseous replacement starts earlier, shortening the recovery time.

For sinus lift procedure, the recommended Bonelike® granulometry is 250-500μm, since it allows osseous intergranular growth followed by neovascularization resulting in elevated osteoconductivity. On average 2 g of Bonelike were necessary for a sinus lift surgery, which meant that 0,5 g of calcium sulphate would be sufficient to stabilize the graft. A good condensation of the graft material is essential so that all the spaces, within the bone defect cavity, are filled preventing air sockets formation. The advantage of using a synthetic graft material essentially relies on its limitless availability and exemption of infectious disease transmission and immune rejection. The resource to autologous bone, the golden standard material in oral surgery, becomes scarce in intraoral localizations, compelling to extra oral donor locations. The procedure becomes thus more complex, requiring an additional surgery, under general anaesthesia, and with increased risks and complications. The resource of the described surgical technique and the use of the stabilized biomaterial with so singular characteristics contributed for post-surgical sinusitis absence, as well as for nonexistence of graft infection. Moreover, the osseous biopsy taken at 9 months postoperatory, the implant setting time, revealed new bone formation surrounding all Bonelike® granules surface thus evidencing the procedure and graft success. In Figure 5 is depicted a detailed clinical procedure for the sinus lift surgery using Bonelike®.

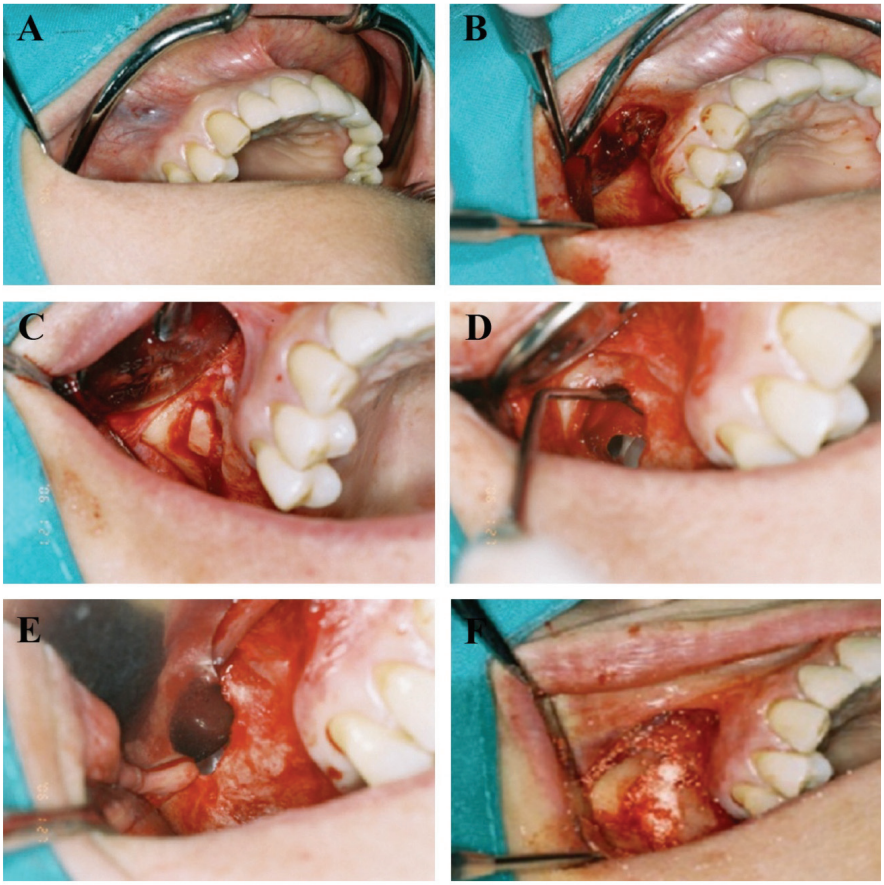

*Figure 5: Maxillary sinus floor elevation clinical procedure. (**A**) Patient's initial situation; (**B**) Rising of the mucoperiostical flap; (**C**) Lateral window to access the sinus; (**D**) Rising of the sinus membrane; (**E**) Socket prepared to receive the graft; (**F**) Graft placement on the socket.*

As control of the clinical procedure, preoperatory, postoperatory and a follow-up 9 months postoperatory X-ray orthopantomograms were performed (Figure 6). No sign of postoperatory graft displacement is observed (Figure 6B). The maxillary osteoregeneration is evident at 9 months postoperatory, without post-surgical sinusitis or infection (Figure 6C).

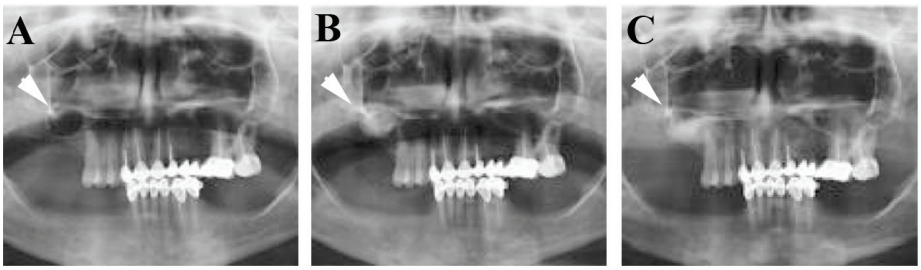

*Figure 6: X-ray orthopantomograms. (**A**) Preoperatory; (**B**) Postoperatory; (**C**) 9 months postoperatory. The asterisk shows the Bonelike® implantation site.*

Figure 7 shows some histological images of the sinus lift study. From the images, Bonelike® demonstrated to be an alternative biomaterial with high superior results when used as a graft material in sinus lift surgeries. New bone formed and osteointegrated to Bonelike® may be observed in Figure 7.

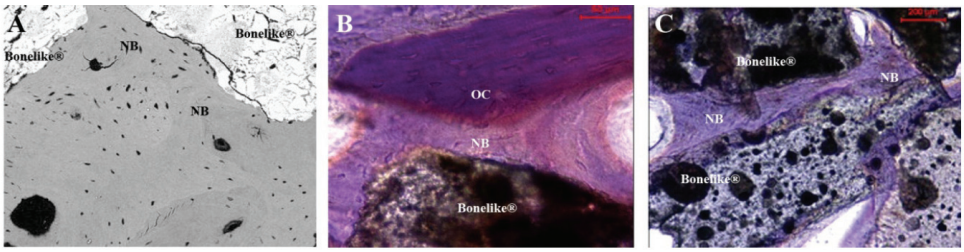

***Figure 7**: Histological analysis. (**A**) SEM image of bone regeneration and Bonelike®- new bone interface; (**B**) Solo-Chrome R staining revealing new bone formed and osteointegrated (**C**) Solo-Chrome R staining showing Bonelike®- new bone interface (NB-new bone; OC-osteocyte). A-magnification 300×; B-magnification 200×; C-magnification 50×.*

5.2 ORTHOPAEDICS SURGERY

Dealing with bone defects is a permanent challenge in orthopaedic and trauma field. Valgus tibial osteotomy is an efficient method of treatment for patients with medial compartment osteoarthritis in varus knees. Autografts, more specifically iliac crest cortico-cancellous graft, is considered the most successful bone graft because of its osteoconductive, osteoinductive and osteogenic properties. However, as previously mentioned autografts possess some drawbacks related to the donor site and requirement of a second surgery [4, 5] To overcome autograft's disadvantages, synthetic bone substitutes morphologically adapted to the host site are starting to be used in several

orthopaedic applications. In this section, the reports of biological responses of human bone tissue to Bonelike® are discussed. Bonelike® was implanted either as a macroporous cylindrical specimen in the lateral aspect of the tibia of patients with medial compartment osteoarthritis of the knee during osteotomy surgery [82], or as a wedge specimen for the correction of valgus knee through open wedge high tibial osteotomies (HTO) [83].

5.2.1 MACROPOROUS CYLINDER

In orthopaedic applications, a synthetic bone graft has to accomplish a perfect controlled balance between porosity and the initial mechanical strength, thus guarantying an optimal scaffold per surgical application upon load bearing while maintaining the structural parameters necessary for host bone ingrowth.

Bonelike® improved mechanical properties, due to the presence of α- and β-TCP in the HAp matrix, assure good results in orthopaedics applications [79]. Therefore, Bonelike® porous cylindrical specimens of 8x10 mm were implanted in the lateral aspect of the tibia of thirteen patients, with a mean age of 54 years old, during osteotomy surgeries for the treatment of medial compartment osteoarthritis of the knee [82]. Implanted cylinders were retrieved at the same time of the removal of the blade plates at 3, 6, 9 and 12 months postoperatory. The biological responses of human bone tissue to porous Bonelike® were assessed by SEM and light microscope evaluation.

The typical microstructure of a Bonelike® macroporous sample is possessed an estimated value of total porosity, based on the determined apparent density, of 90%. The majority of total macroporosity consisted of open and interconnected pores [82].

The clinical study included ten women and three men, with a mean age of 54 years (range 48 to 66 years). In these patients the left side (knee) was most frequently affected. The inclusion criteria were active patients with medial compartment osteoarthritis of the knee, with no more than 70 years, varus deformity of less than 15° and no associated inflammatory disease. It was also not consider mild-to-moderate patello-femoral osteoarthrosis as a contraindication for high tibial osteotomy (HTO).

To evaluate the biological behaviour of the Bonelike® to regenerate bone defected areas, a 8 mm diameter hole was created in the lateral aspect of the tibia, 3 cm distal to the entry point of the screws. A porous cylinder with exactly the same size was press-fitted there in order to fill the cavity as shown in Figure 8. A second surgical procedure allowed collecting samples at the pre-determined dates (3, 6, 9 and 12 months) to proceed with the subsequent histological study, with the same approach, and using a 12 mm trephine to cut the cortical bone around the specimens.

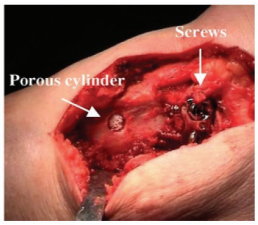

Figure 8: Bonelike® macroporous cylinders were pressed fitted into an 8 mm hole, 3 cm distal to the entry point of the screws.

SEM analysis of the retrieved samples (Figure 9), and histological analysis (Figure 10) revealed no collapse of the Bonelike® cylinders by microfracture, and direct apposition of bone on the Bonelike® surface was found in all tissue sections with no fibrous tissue interface. The pores located in the periphery were the first to be filled with bone as envisaged from the histological surveys after 3 months of implantation. A centripetal bone colonization from the periphery to the middle of the cylinder was observed but in continuity with the surrounding host bone. The percentage of bone new coverage on the porous Bonelike® internal surface increased with implantation time.

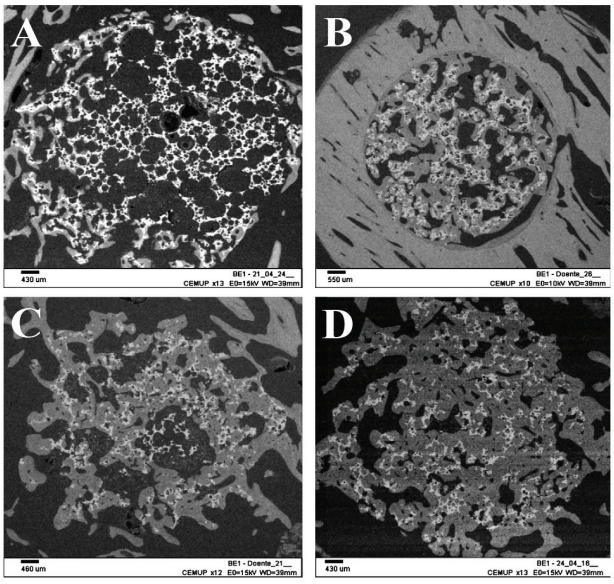

*Figure 9: Back-scattered SEM images of implanted macroporous Bonelike®. Bone centripetal growth after (**A**) 3 months, (**B**) 6 months, (**C**) 9 months and (**D**) 12 months of implantation.*

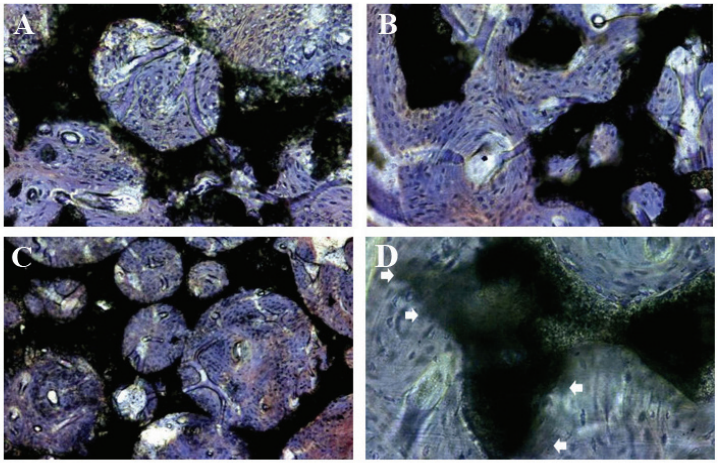

Figure 10: Histological images (Solo-Chrome R staining) of macroporous Bonelike® samples retrieved after 6 (A), 9 (B and D) and 12 (C) months of implantation (magnification 100×·).

The percentage of bone new coverage on the porous Bonelike® internal surface increased with implantation time. Histomorphometry analysis revealed a 100% of penetration for all implantations periods except for 3 months where bone could only be seen in the periphery region. Regarding the percentage of the area covered by new bone calculated from 2D histological sections, a value of 53%(±15%) was achieved for 6 months, and no statistical differences were found between the values obtained for 9 and 12 months (76% ±12% and 88%±9 %, respectively) of implantation since bone was found to penetrate the channels and fill up most of the channel spaces. Significant Bonelike® degradation was not reported in the short implantation periods, while clear surface dissolution was visible after 9 months of implantation (Figure 10D).

Therefore, the present clinical study confirmed other previous studies that had shown, Bonelike® was rapidly osteointegrated after implantation, exhibiting controlled balance between porosity and the initial mechanical strength for this kind of orthopaedic application [82].

5.2.2 WEDGE FOR HIGH TIBIAL OSTEOTOMY

In orthopaedics applications, autologous iliac crest cortico-cancellous graft is considered the most successful bone substitute because of its osteoconductive, osteoinductive and osteogenic properties. However, autografts showed some drawbacks related to the donor site and invasive harvest procedure [4, 5]. Synthetic bone substitutes morphologically adapted to the host defect site are starting to be used in

several orthopaedic applications in order to overcome the disadvantages of the autografts.

In fact, 3D porous scaffolds seem to be a good option for opening wedge HTO, as they offer a framework for new bone tissue formation and anchorage by providing surface and volume, which will allow cell ingrowth and an accurate cell distribution throughout the porous structure [111-114]. Particularly pre-shaped wedges custom-designed for valgus knee correction simplified the HTO surgical procedure, as well as provided some mechanical stability.

Bonelike® with improved mechanical properties, as well as excellent biological behaviour, as established by macroporous cylindrical specimens, become an apparent suitable synthetic bone graft for wedges preparation.

For wedge preparation, the Bonelike® composite powders were firstly uni-axially pressed at 120 MPa to obtain dense block shaped compacts. Then, the blocks were then machined into an edge shape with porous structure distribution, according to Figure 11. Finally, the obtained porous Bonelike® wedges were sintered at 1300 ºC during 1 h [83]. The dimensions of the wedges, required for this particular case, were: 40 x 19 mm, the tip and the base height were 4 mm and 12 mm (Figure 11).

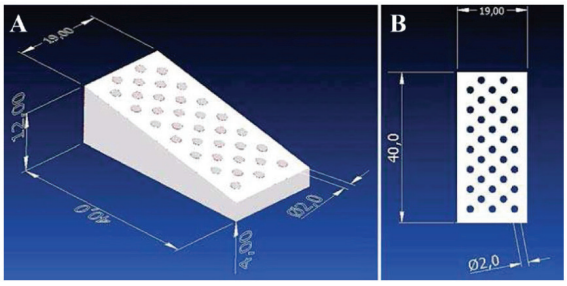

Figure 11: Bonelike® wedge. (A) Isometric view; (B) Top view. The length of the edge is 40 mm, the thickness is 19mm and the tip and base height is 4 and 12 mm, respectively. The space between porous is 2.0 mm.

The preoperatory evaluation, according to the standard protocol *International Knee Score* (IKS), of a 58 years old male patient, revealed bilateral genum varum, with quadriceps atrophy, good range of motion, small amount of intraarticular fluid, positive valgus stress test, but negative Mc Murray and anterior drawer test. The radiographic preoperatory examinations showed bilateral osteoarthrosis, mainly in the medial compartment and a varus of 6 º in the left and 10 º in the right knee (Figure 12A-C).

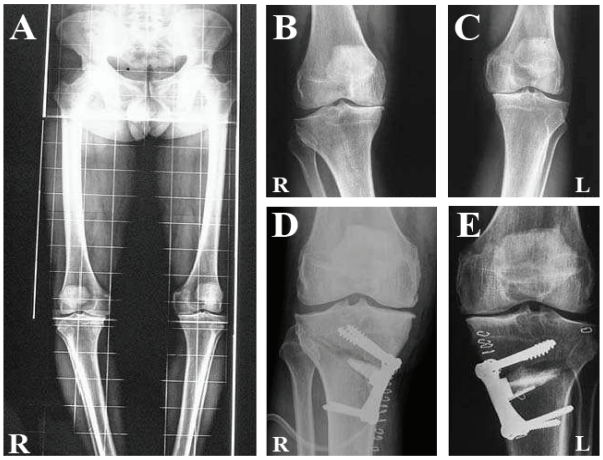

Figure 12: Radiographic examination. (A) Preoperatory general X-ray; (B) Preoperatory X-ray of the right leg showing a 10º anatomical varus; (C) Preoperatory X-ray of the left leg showing a 6º anatomical varus; (D) Postoperatory X-ray after 8 weeks of right knee treated with Bonelike® wedge; (E) Postoperatory X-ray after 8 weeks of left knee treated with commercial HAp/β-TCP.

Therefore, an opening wedge osteotomy was planned to obtain 2 or 3 ° of hypercorrection in the alignment of both knees, which is 9 or 10 ° valgus. A comparative study was performed employing the above mentioned Bonelike® wedge and a commercially available HAp/β-TCP biphasic wedge.

Firstly, the patient was submitted to a valgus osteotomy in the left knee with addition of a 12° medial wedge of a commercial HAp/β-TCP biphasic material (Figure 12E). Eight months later the same surgical procedure was applied to the right knee using the Bonelike® (Figure 12D). In both the cases, the medial wedge opening side was stabilized by means of a Puddu plate and screws to avoid subsequent displacement of the wedge (Figures 12D and 12E).

The postoperatory period was normal, with no signs of inflammatory reaction. At two months postoperatory, radiological evaluation indicated signs of fusion at the osteotomy site and good quality integration of the implanted wedges. Partial weight bearing was allowed at 4 months. The clinical outcome was an achievement of correction of 10° valgus on the left knee, 6° valgus on the right knee and an overall favourable ISK score in both knees [83].

In the previous section, histological histomorphometric analysis and SEM analysis demonstrated Bonelike® improved osteoconduction and resorption rate concomitant

with new bone formation rate for orthopaedics application. The presented clinical report, opened-up the orthopaedic areas of application of Bonelike® to opening wedge HTOs.

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