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Upgrading calcium phosphate scaffolds for tissue engineering applications

Sandra Sánchez-Salcedo[a], Daniel Arcos[b] and María Vallet-Regí[c]

Dpto. Química Inorgánica y Bioinorgánica. Facultad Farmacia.
Universidad Complutense de Madrid
Plaza Ramón y Cajal, s/nº
28040-MADRID
[a]sansanch@farm.ucm.es, [b]arcosd@farm.ucm.es, [c]vallet@farm.ucm.es

Abstract

The research on ceramic scaffolds for bone tissue engineering is, nowadays, one of the newest and most attractive topics in the field of materials for biomedical applications.

These scaffolds are aimed to provide supporting or even enhance the reparative capacity of body. Biphasic calcium phosphates (BCPs) and silicon doped BCP are very interesting candidates to be used as materials for scaffolds fabrication in bone tissue engineering. BCPs and silicon doped BCP consist of a mixture of hydroxyapatite (HA) and β-tricalcium phosphate (β-TCP) or HA and α-tricalcium phosphate (α-TCP), respectively. For the regenerative purposes BCPs show better performance than HA because of the higher solubility of β-TCP compound, which facilitate the subsequent bone ingrowth in the implant. On the other, silicon doped BCP involve silicon that substituted into the apaptite crystal lattice for phosphorous with the subsequent charge imbalance. HA/α-TCP based bioceramics exhibits an important improvement of the bioactive behaviour with respect to non-substituted apatites. This work reviews the procedures to synthesise and fabricate scaffolds based on HA/β-TCP and silicon stabilised HA/α-TCP. Special attraction has been paid in the different synthesis methods and to the shaping of final scaffolds. By knowing the scaffold features at the crystallinity and macrostuctural level, the biocompatibility and clinical performance can be better understood, which will be also considered in this review.

Keywords: Biphasic calcium phosphate, biocompatibility, tissue engineering, silicon

Introduction

Calcium phosphate (CaP) bioceramics represent one of the main inorganic material families in the field of dental and orthopedic reconstructive medicine. The story of the successful CaP implantations began in 1920, when Albee used a CaP reagent (described as `triple calcium phosphate') for repairing a bone defect in a human patient [1]. However, the modern development of CaP based biomaterials occurred in the early 1980s, with the commercialization of synthetic hydroxyapatite (HA) and β-tricalcium phosphate (β-TCP) for dental and bone tissue applications. In this sense, the work carried out by Jarcho *et al* played a fundamental role in the 'lab-to-industry' transfer of knowledge [2-5].

Now a days, the general requirements for ideal bone grafts CaP implants establish that should exhibit pores of several hundred of microns, biodegradation rate comparable to the formation of bone tissue (i.e. between a few months and about 2 years) and the sufficient mechanical stability [6]. HA and TCP (both, α and β polymorphs) do not fulfill these requirements and some clinical failures have occurred as a consequence of non-appropriated biodegradability kinetic, which eventually will involve a disadvantage to the host tissue surrounding the implant. For instance, some implants made of calcined HA to reconstruct mandibular ridges defects have resulted in high failure rate in human clinical applications [7]. In order to avoid this problem, the use of granular instead of block forms of HA was suggested [8], although HA exhibit problem due to lack of biodegradability, independently of the implant form. On the other hand, β-TCP ceramics have been developed as a biodegradable bone replacement and commercially available as, for instance, ChronOS^{TM}, Vitoss^{TM}, etc. [9]. However, when used as a biomaterial for bone replacement, the rate of biodegradation of TCP has been shown to be too fast. In 1988, Daculsi et al. [10] thought that the presence of an optimum balance of stable HA and more soluble β-TCP should be more favourable than pure HA and β-TCP. Due to the biodegradability of β-TCP component, the reactivity increases with the β-TCP/HA ratio. Therefore, the bioreactivity of these compounds can be controlled throught the phase composition. The main advantage respect to other non soluble calcium phosphates is that the mixture is gradually dissolved in the human body, acting as a stem for newly formed bone and releasing Ca^{2+} and PO_4^{3-} to the local environment [11]. In vivo tests have confirmed the excellent behaviour of BCP (biphasic calcium phosphate) concerning the biodegradability rate [12-15].

Since Nery *et al* [12] termed for the first time a CaP as BCP, to describe a mixture of β-TCP and HA (erroneously identified as TCP in previous works [16]) many advances have occurred in the BCP field. The works carried out by Daculsi *et al*. [14, 17] impelled the commercialization of BCP and currently can be found as trade marks like Triosite™, HATRIC™, Tribone™, etc. [11]. Nowadays, BCP are clinically used as an alternative or as an additive to autogenous bone for dental and orthopaedic applications. Implants shaped as particles, dense or porous blocks, customized pieces and injectable polymer-BCP mixtures are common BCP based medical devices. Moreover, research is in progress to enlarge the clinical applications to field of scaffolding for tissue engineering [18,19] and carriers loading biotech products [20,21].

This work reviews the main strategies for BCP synthesis, as well as its implication in the implants biocompatibility. Moreover, the processing methods that determine the final shape and porosity of the devices are also reviewed and considered in terms of capability to act as bone filler, cement, scaffold or even as a carrier system of biotech products. Finally, the role of the silicon incorporation for the synthesis of Si-stabilized HA/α-TCP is also critically considerd as potential osteoinductive material.

BCP based bone implants: synthesis strategies

Until recently, BCP ceramics had only accidentally been encountered during the synthesis studies of pure HA [22] or pure β-TCP [23]. Nowadays, there are several strategies concerning solid state synthesis, wet chemical methods and alternative techniques that lead to BCP with controlled chemical and microstructural characteristics.

Biphasic HA/β-TCP material can be prepared by solid state methods such as *physical mixing* of pure HA and β-TCP phases [24,25], or by the typical *ceramic method* through the thermal treatment of different raw materials at temperatures above 1000ºC. For instance, Yang et al. [26] carried out the synthesis of BCPs series by a process based on solid state reaction of brushite with calcium carbonate. After establishing a nominal BCP composition through the stoichiometric amounts of precursors, these authors concluded that pure HA and β-TCP are obtained at 1200 and 900ºC, respectively. In order to obtain the designed HA/β-TCP ratio, the thermal treatment must vary in the range of 1000 to 1100ºC. Since the composition has a larger HA content when the mixture of raw materials sintered at higher temperature, BCPs with controlled phase compositions can be obtained by adjusting the sintering temperature from 1000-1100 ºC. Depending on the kind

precursors and thermal treatment, solid state reactions can lead not only to BCPs of different HA/β-TCP ratios but also to different phases. Rao et al. [27] were able to obtain BCPs by solid state reaction of tricalcium phosphate and calcium hydroxide at 1000ºC when the molar ratio TCP:$Ca(OH)_2$ were 3:1 and 3:1.5. Pure HA is obtained with 3:2 or 3:3 ratios for the same thermal treatment. On the contrary, higher contents of $Ca(OH)_2$ result in the formation of CaO.

Fig. 1 schemes the most common strategies for BCP synthesis. Although solid state methods are suitable strategies to obtain BCP, wet chemical methods are the most common synthesis routes for this purpose. Wet chemical methods shows two principal routes to obtain BCPs: (1) *hydrolysis* of amorphous calcium phosphates (ACP), octacalcium phosphate (OCP), dicalcium phosphate dihydrate (DCPD) and α-TCP; and (2) *precipitation methods*, that entail the presence of a source of calcium, such as calcium acetate, calcium hydroxide or calcium nitrate and a source of phosphorous, such as diammonium hydrogen phosphate, ammonium dihydrogen phosphate, phosphoric acid or a mixture of dipotassium hydrogen phosphate/potassium dihydrogen phosphate.

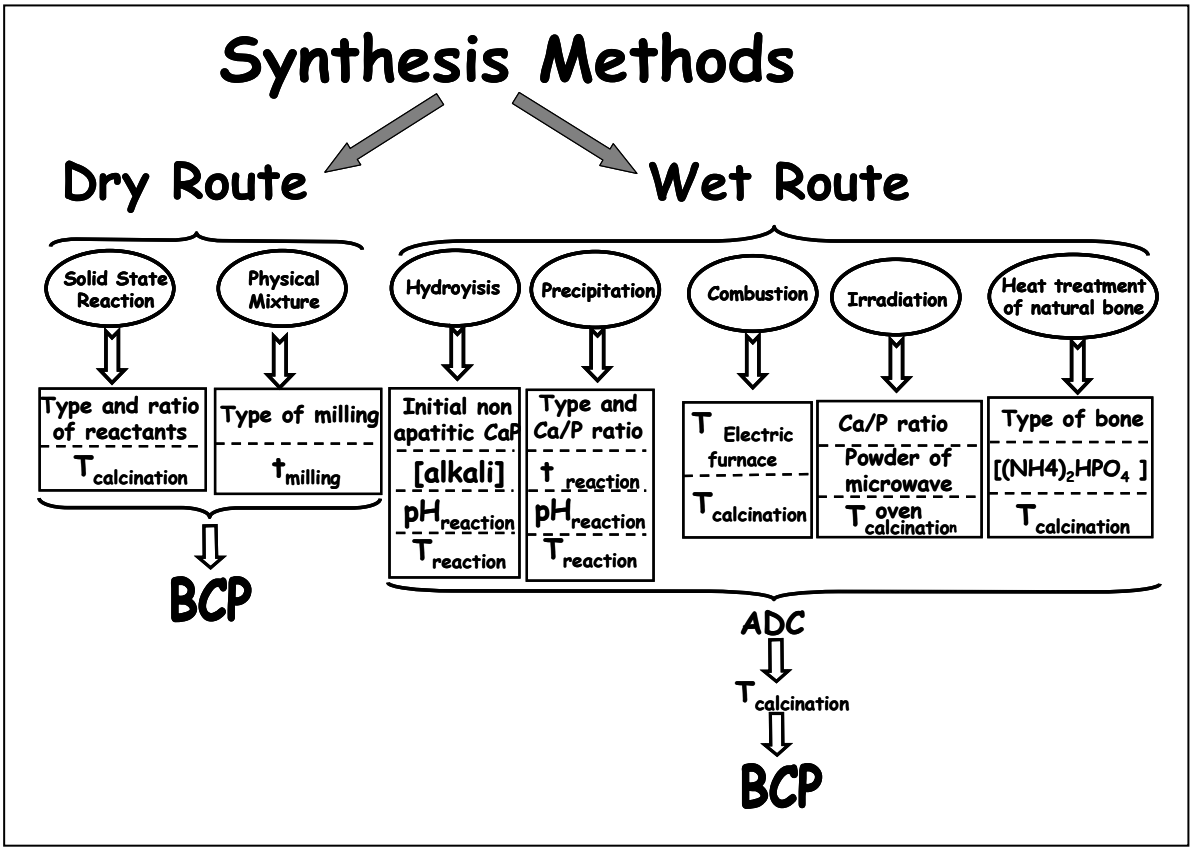

Figure 1. Synthesis methods of BCP bioceramics.

Both strategies lead to calcium deficient apatite (CDA) precipitation with a Ca/P ratio ranging between 1.5-1.67. The subsequent thermal treatment gives rise to CDA decomposition into BCP (see Eq. 1), forming more intimate HA-βTCP mixtures compared with those obtained by mechanical mixing methods [28]. The presence of β-TCP in BCP ceramics is observed when CDA is treated above 700ºC, whereas thermal treatment above 1125ºC results in β-TCP transformation into α-TCP [30].

$$Ca_{10-x}(PO_4)_{6-x}(HPO_4)_x(OH)_{2-x} \rightarrow (1-x)Ca_{10}(PO_4)_6(OH)_2 + 3xCa_3(PO_4)_2 + xH_2O \quad (1)$$

The CaP hydrolysis method allows controlling the reaction through parameters such as alkali concentration, pH, temperature and the raw CaP chosen. For instance, LeGeros [24] described the synthesis of CDA by hydrolysis of OCP in Ca-containing solutions at 60 to 100ºC and initial pH solution of 7. The so obtained CDA showed the typical acicular morphology of precipitated HA, although some crystal-chemical differences such as a-axis larger than that of pure HA, due to HPO_4^{2-} incorporation, could be identified. Bouler et al.[28] prepared CDA from hydrolysis of DCPD in boiling aqueous NaOH solutions of several concentrations and confirms the marked influences of the alkaline solution on the stoichiometry of the formed CDA and consequently, on the HA/β-TCP ratio of the final BCP. Therefore, correlation between the pH measured at the end of the hydrolysis and the final BCP composition allows us to predict and to control *in situ,* the stoichiometry of the apatite formed. However, CDA and BCP obtained using NaOH present Na^+ in their structure. In order to avoid this fact, Dorozhkina et al. [29] carried out the obtention of BCP using as alkali solution KOH and found no incorporation of K^+ ions into the crystal lattice of apatite. Moreover, a mechanism for the transformation of CDA into HA and β-TCP were purposed. The mechanism implies the OH^- and Ca^{2+} ions diffusion over the distance of 1-2 cristallites (19-38 nm), in order to provide the transformation. Since that, the diffusion of OH^- was 0.18-0.72 s and diffusion of Ca^{2+} was 1-4 h at 1000ºC, the thermal treatment used of 1050ºC/4 h to provide the transformation of CDAs obtained in this research, was enough to give rise a biphasic material which consist of a mixture of HA and β-TCP.

TenHuisen et al. [31] prepared CDA by hydrolysis of α-TCP and determined that the reaction mechanism occurs in two steps. The initial step, with a high initial pH values, was a result of slight compositional inhomogeneities attributable to synthesis conditions initially employed. The elevated pH also resulted in a reduction of the rate of hydrolysis. The second reaction step resulted in the bulk of hydrolysis. The activation energies for these steps were determined indicating a nucleation and growth mechanism. The microstructure of the resulting CDA differed greatly from that produced by the DCPD-tetracalcium phosphate reaction at equivalent temperatures. In particular, crystallites are much larger, resulting in lower surface areas, and the hydroxyapatite formed is more crystalline than that produced by reaction of DCPD with tetracalcium phosphate at equivalent temperatures. The author attributed the difference to the slower rate of TCP hydrolysis combined with the absence of the formation of ACP as an intermediate.

BCP synthesis through CDA *precipitation methods* requires an accurate control on both synthesis parameters (pH, temperature, stirring/maturation time, etc.) and reactants stoichiometry. BCPs synthesis has been carried out from the homogeneous mixture of $Ca(NO_3)_2$ and $(NH_4)_2HPO_4$ and posterior heat treatment between 800-1000ºC [32,33]. Small variations of precursors Ca/P ratios lead to great changes in CDA calcium deficiency and the subsequent BCP composition variations after thermal treatment. Higher β-TCP/HA ratios are obtained insofar the Ca deficiency increases in the precursor CDA. Moreover, the amount of calcium deficiency in CDAs has an important influence on the thermal stability. Our research group have carried out the synthesis by controlled crystallization method of CDAs with nominal formula $Ca_{10-x}(HPO_4)_x(PO_4)_{6-x}(OH)_{2-X}$ for x= 0.58 and 0.73. The transformation of CDA on HA and β-TCP begins at 750 ºC, although, the total transformation into the BCP ceramic finished at 800ºC and 900ºC, for CDA with x= 0.58 and x= 0.73, respectively. Figure 2 shows the phase content evolution as a function of the thermal treatment and evaluated by XRD diffraction patterns, subsequently analyzed by full profile refinement methods. The application of XRD for quantitative phase analysis is a very useful tool, to control the BCPs composition. This analysis is very important in methods like precipitation synthesis, where the phase composition is highly dependent on small stoichiometric changes. In this sense, our research group have applied the Rietveld refinement of powder XRD patterns to BCP quantification of phases, and demonstrated that this method is a useful and reliable tool to determine and control the phase composition as a function of the CDA calcium deficiency [34].

Choosing appropriated salt reactants is also mandatory to control the final composition. It must be taken into account that the apatite structure admits numerous ionic substitutions into the crystalline structure. Therefore, the presence of spectator ions in the solutions can modify the CDA stoichiometry and the final BCP phase composition. For this reason, several authors have carried the synthesis of BCP from $Ca(NO_3)_2{\cdot}4H_2O$ and $(NH_4)_2HPO_4$. The control of the final product purity can be achieved using spectator ions such as NO_3^- and NH_4^+, with very low incorporation degree into the crystalline apatite structure [35,36].

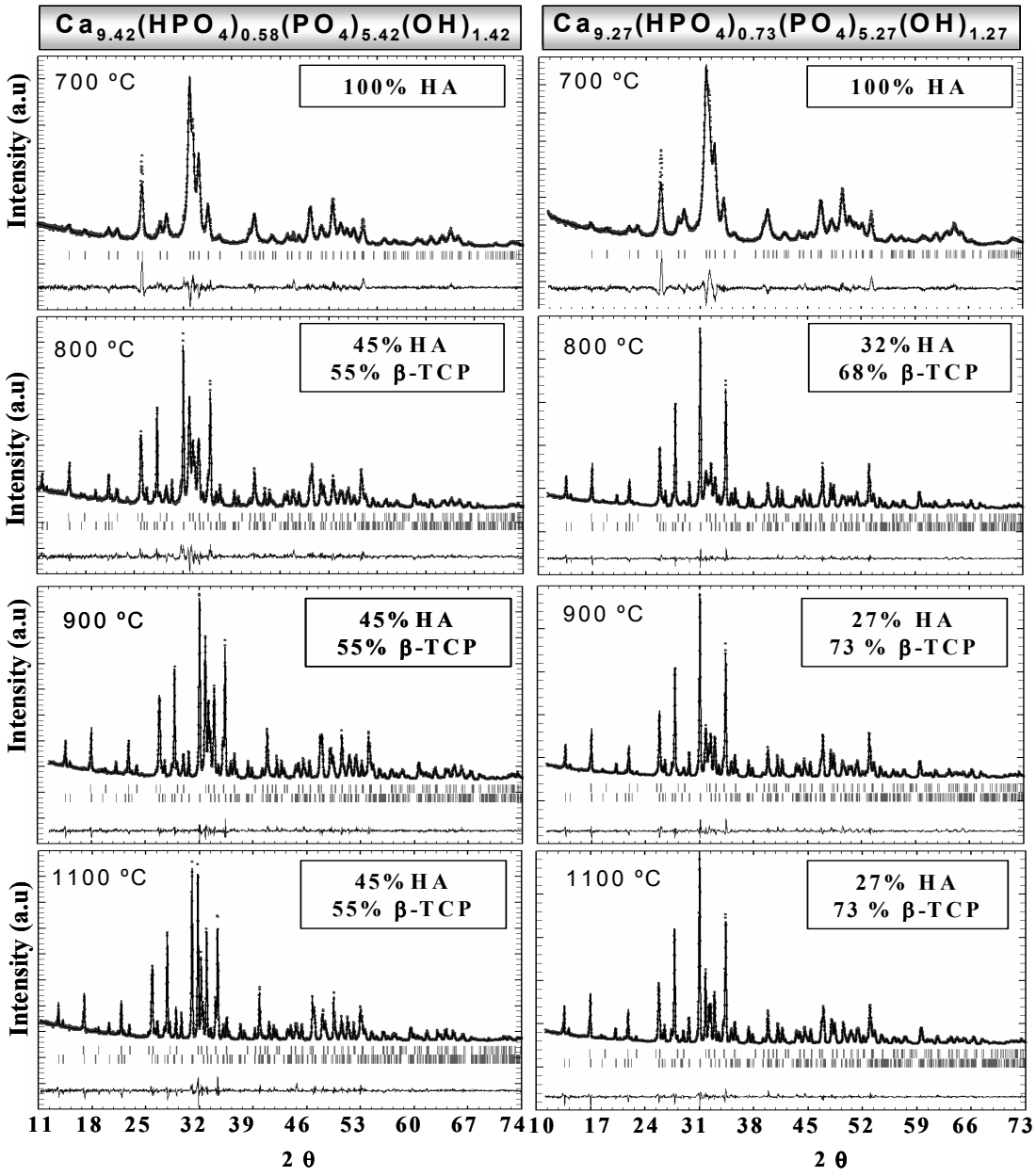

Figure2. Powder X-ray diffraction patterns for two CDAs:$Ca_{9.42}(HPO_4)_{0.58}(PO_4)_{5.42}(OH)_{1.42}$ and $Ca_{9.27}(HPO_4)_{0.73}(PO_4)_{5.27}(OH)_{1.27}$ samples treated at 700, 800, 900 and 1100ºC. The vertical lines mark the positions of Bragg peaks for an apatite-like and β-TCP phases.

Other alternative strategies have been also employed to synthesise BCPs. The *self-propagating combustion method* was shown to be an alternative and quick way of synthesizing submicron (0.45 μm) powders of BCPs after calcination at 1150ºC [37]. A novel simulated body fluid (SBF) solutions were used for the synthesis, since that BCP contains small concentrations, at the ppm levels similar to the chemical composition of natural bones, of elements such as Mg, Cl, Na, K, Fe, Zn and Cu. *Microwave irradiation* allows the formation of in situ BCP ceramic altering the HA:TCP ratio easily to form BCP of desired properties with no decomposition process. This method suggests the possibility of producing BCP in situ, without stabilizing TCP agents and a final product without impurities [38]. The *heat treatment of natural bone* is a simple process to prepare different HA/β-TCP ratios of bioceramics with a natural bone porous structure [39]. The elimination of the organic substance is carried out by a burning process. After the addition of different quantities of an ammonium phosphate $(NH_4)_2HPO_4$ solution, the bone is heated at 600 to 900 ºC to transform the biological HA phase into a β-TCP/HA biphasic bioceramic.

The Biocompatibility of BCP implants

Between 1920 and 1975, only a few researchers reported on the use of CaP materials to repair bone defects or periodontal defects [1,16,40]. Thereafter, LeGeros [41] identified the so called "tricalcium phosphate" used by Nery [16] as a mixture of 20%β-TCP and 80%HA. Since then, different mixtures of β-TCP and HA denoted as 'biphasic calcium phosphate' have been described as highly biocompatible materials and clinically used. Nowadays, BCP bioceramics used in clinical practice are available in (1) *blocks*; (2) *granulates* and (3) *injectable particles* within a polymer carrier. The biocompatibility behaviour of BCPs depends on the way that this material is implanted. The implant shape significantly influences the behaviour of BCPs when they are in contact with natural tissues, particularly their capacity to be reabsorbable, biodegradable and non cytotoxic.

Porous blocks have been widely tested as a bone substitute in the clinical practice. The principal research aim dealing with porous BCP blocks is the *osteoconduction* and the subsequently *osseointegration* of the implant*, i.e.*, BCPs must be able to guide new bone formation and form a tight bond with the newly formed bone. Among the CaP, BCP materials are very interesting due to the reactivity can be controlled through the phase composition, *i.e.,* the bioreactivity increases with the β-TCP/HA ratio, due to the higher

solubility of the β-TCP component. Therefore, the formation rate of new bone is closely related and can be controlled with the rate of bioreabsorption of the BCP implant. In this sense, Daculsi et al. [42] observed that, after six months of *in vivo* implantation, newly formed bone was present on series β-TCP/HA with weight ratios of 15/85, 35/65 and 85/15. Their results demonstrated that the dissolution of BCP ceramic implants was influenced by the β-TCP/HA ratios: the higher β-TCP/HA ratio, the greater the abundance of the newly formed apatitic crystals is observed.

Besides BCP composition, another important and difficult BCP blocks requirement is to have an appropriate porosity. LeGeros et al. [43], using sintered blocks with macroporosity of about 150 to 200 μm, have demonstrated that two concurrent and complementary mechanisms may occur after implantation. In the micropores, the dissolution of BCP crystals release calcium and phosphate ions to the biological fluid, causing the precipitation of new apatite crystals associated with proteins present in the biological fluid. In the macropores, the reabsorption occurs simultaneously with the invasion of osteoblastic cells leading to bone formation.

More recently, it has been observed that macroporosity would play a key role in cellular phenotype expression and the osteogenesis process. It has been observed that macroporous BCPs scaffolds induced osteogenesis and thus accelerate bone formation in the pore region of the ceramic after implantation [44-46]. This fact point out that the blocks macroporosity is not only involved in osteoconductive process but also in the osteoinductive mechanism.

Several research groups have reported on the in vivo evaluation of commercially available BCPs blocks [47-54]. Specially one MBCP™ or Triosite™, which is a micro macroporous biphasic calcium phosphate with a 60:40 HA/β-TCP ratio and 40-50 % of macropores ranging from 300 to 600 μm in size. The *in vivo* evaluation of MBCP™ as bone graft substitutes instead of autogenous bone and the influence of the macroporosity on the osteoconduction of BCP ceramics have been widely researched. MBCP™ showed an excellent bone bonding capacity, absence of allergenicity and can be used as a bone graft substitute in orthopaedic and dental surgery. Gauthier et al. [55] concluded that MBCP™ scaffolds with 565 μm and 40% macroporosity percentage are the more adequate, providing mechanical efficiency and preserving optimal bone ingrowth.

In the field of tissue engineering, the *in vitro* tissue formation on/in porous blocks are required the implantation in the body. For that reason, in vitro cell behaviour assays have

acquired and added importance in the last years [54,56]. The in vitro evaluation of osteoblasts and osteoclasts onto dense blocks of HA, BCP and β-TCP with very similar microstructural and surface properties, showed significantly higher osteoblast proliferation and more adequate osteoclasts reabsorption in BCPs than the other CaP ceramics [57,58]. Therefore, BCP blocks seem to be better candidates as scaffolds for bone tissue engineering. (Fig. 3)

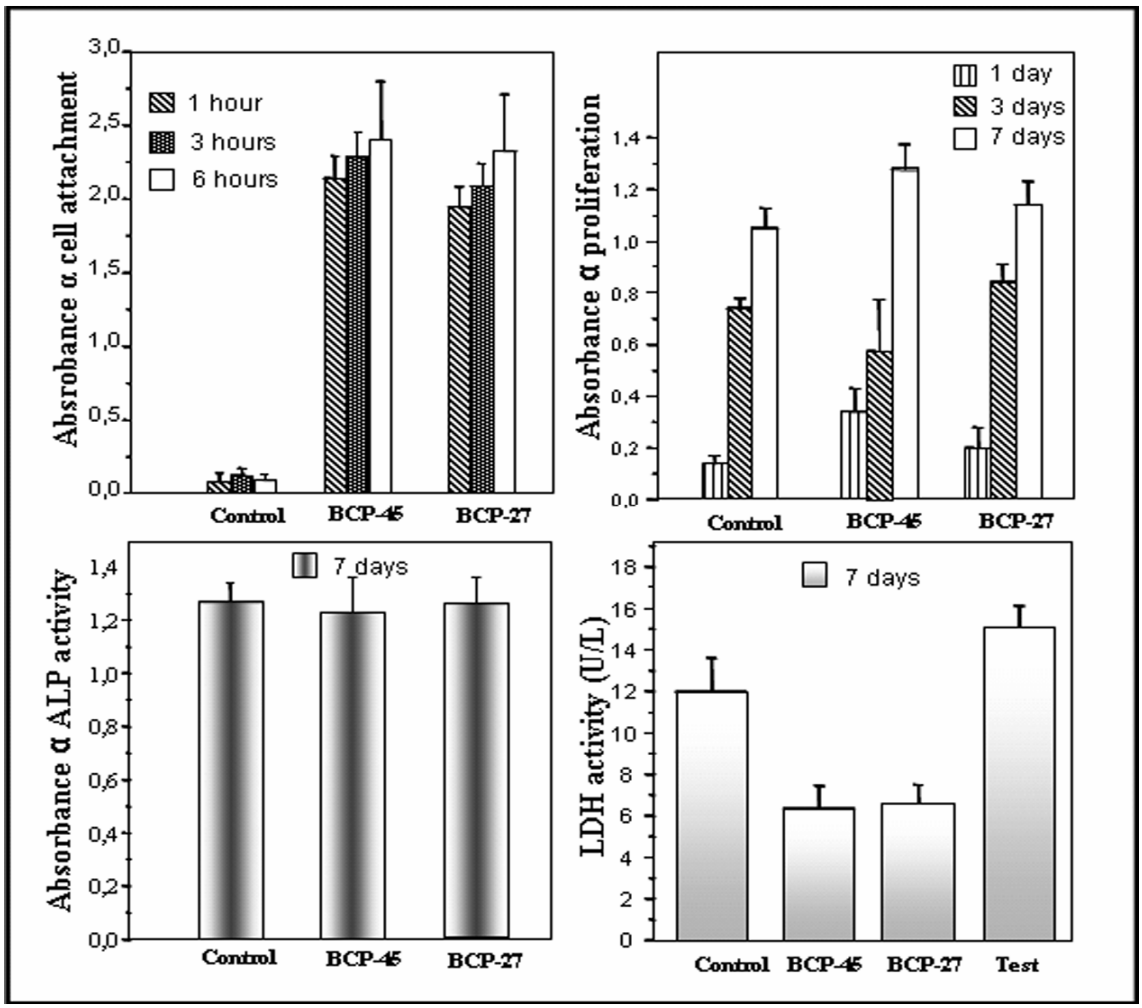

Figure 3. Biocompatibility cell culture tests onto BCP-45 (45%HA-55%β-TCP) and BCP-27 (27%HA-73%β-TCP); (a) Osteoblast attachment at 1, 3 and 6 hours of culture. (b) Osteoblast proliferation at 1, 3 and 7 days of culture (c) ALP relative level at 7 days culture. (d) LDH activity at 7 days of culture. Bar labeled as "test" corresponds to the LDH measured after lysing 10.000 cells.

The properties of a scaffold such as, composition, microstructure and surface chemistry are crucial variables in order to determine the capacity to absorb proteins onto

ceramic surface, such as laminin, vitronectin and fibronectin [56,59]. This ability is very desirable due to the cascade of events, which result in functional bone bonding, is initiated by the protein adhesion and followed by attachment, proliferation and differentiation of bone cells. In this sense, BCP ceramics show excellent properties to improve proteins adhesion [57,60], leading to an easy osteoblast cell development in contact with the BCP surface (figure 4).

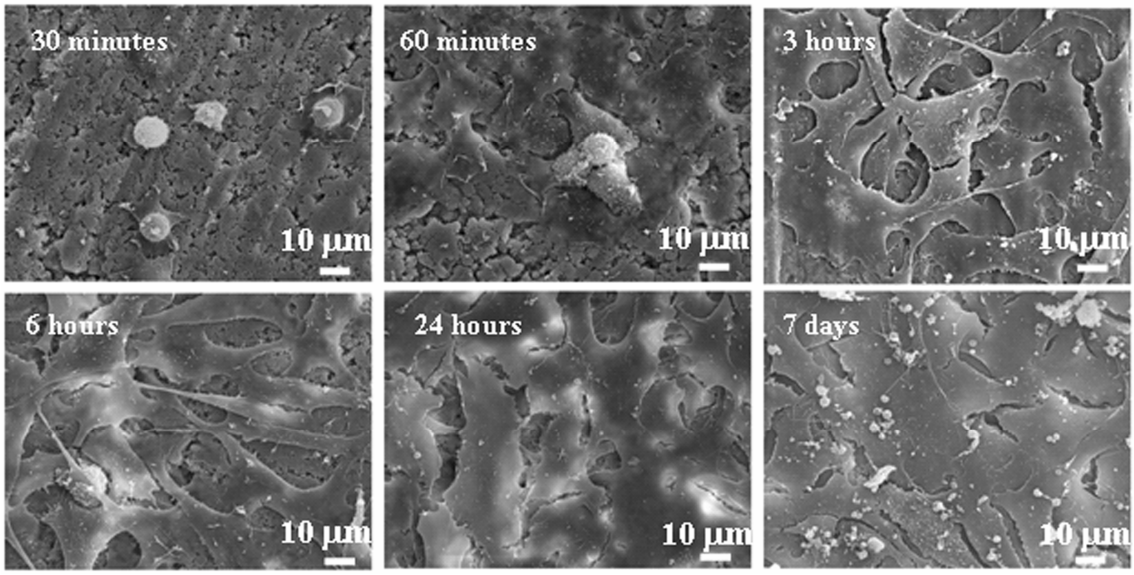

Figure 4. SEM micrographs of osteoblastic development onto BCP-27 (27%HA-73%b-TCP) between 30 min. and 7 days.

Another widely developed field is the application of BCP as *granulates*. Granulates are principally an attractive approach as bone substitute due to the high surface area cell-biomaterial interaction. This advantage would improve the basic mechanism formation of bone-biomaterial bond and their effect on bone ingrowth [61]. In this sense, BCP granulates, with size smaller than 600 μm were evaluated and they showed a good *in vivo* biocompatibility of the BCP material due to the absence of inflammatory infiltrate and the bone defects were completely filled by a tissue with the macroscopic features of mature bone [62,63]. However, granules have been reported to be difficult to handle and keep in place after implantation [64].

Another alternative for BCP implantation are particles included within injectable bone substitutes (IBS). IBS consist of a mixture of BCP grains with different particle sizes, commonly smaller than granulates in order to obtain a rheologically suitable paste along

with hydrosoluble polymer. Unlike porous blocks and granulates, IBS have been developed from the point of view of non-invasive surgery.

BCP particles with granulometries in the range of 10-500 μm have been assayed *in vivo* [65,66]. The reported studies have indicated that small BCP particles in the range 10-20 μm and 40–80 μm provided the best bone ingrowth, with a higher resorption/degradation rate in conjunction with stronger early inflammatory reactions. On the other hand, particle sizes of 200–500 μm showed significantly inferior bone-bonding than 10-20 μm powders, whereas they had provided better ingrowth kinetics than 80–100 μm powders. Therefore, inflammatory response, bone ingrowth, and BCP degradation were different according to the grain size. Thus, the more suitable mixture would consist of the smallest BCP particles, which appeared to provide an inflammatory response that promote bone ingrowth and large BCP particles for bone bonding.

BCP scaffolds for tissue engineering. Processing strategies

Tissue engineering offers a promising new approach to regenerate diseased or injured tissues such as bone [67]. For that issue, three dimensional biocompatible porous scaffolds with a highly interconnected porosity are designed in order to allow cell migration, vascularization and diffusion of nutrients [68,69]. Figure 5 shows thestages involved in a bone tissue engineering process. Pore diameters must exhibit values of hundred microns in size [56,70,71] and a number of designed pore interconections to verify in the shortest possible time a bioresorption of the scaffold and the subsequent new bone formation (Table 1). Scaffolds should have a network of interconnected pores where more than 60 % of pores should have a size ranging from 150 μm to 400 μm and, at least 20 % should be smaller than 20 μm.

Table 1. Hierarchical pore size distribution that an ideal scaffold should exhibits [76].

Pore size of a 3D-scaffold	Biochemical effect/ Function
> 1 μm	Interaction with proteins Responsible for bioactivity
1 - 20 μm	Type of cells attracted Cellular development Orientation and directionality of cellular ingrowth
100 -1000 μm	Cellular growth Bone ingrowth Predominant function in the mechanical strength
> 1000 μm	Implant functionality Implant shape Implant esthetics

Nowadays, in order to upgrade the requirements of a scaffold for bone regeneration, many shaping methods have been developed. Our research group has developed CaP based scaffolds with hierarchical pore structure, throught the introduction of porogens [72]. The mixture of appropriate amount of these organic substances with calcium phosphate powder dispersed in a slurry, via gel casting method combined with multiple tape casting method, yielded scaffolds with hierarchical pore structure (functionally graded porosity) when the green pieces were sinterized at 1100ºC. Final pieces showed interconnected pores that increase from interior tapes, towards exterior tapes with a main graded interconnected porosity from 1.6-3.6 μm to 20-51.5 μm of internal and external tapes, respectively.

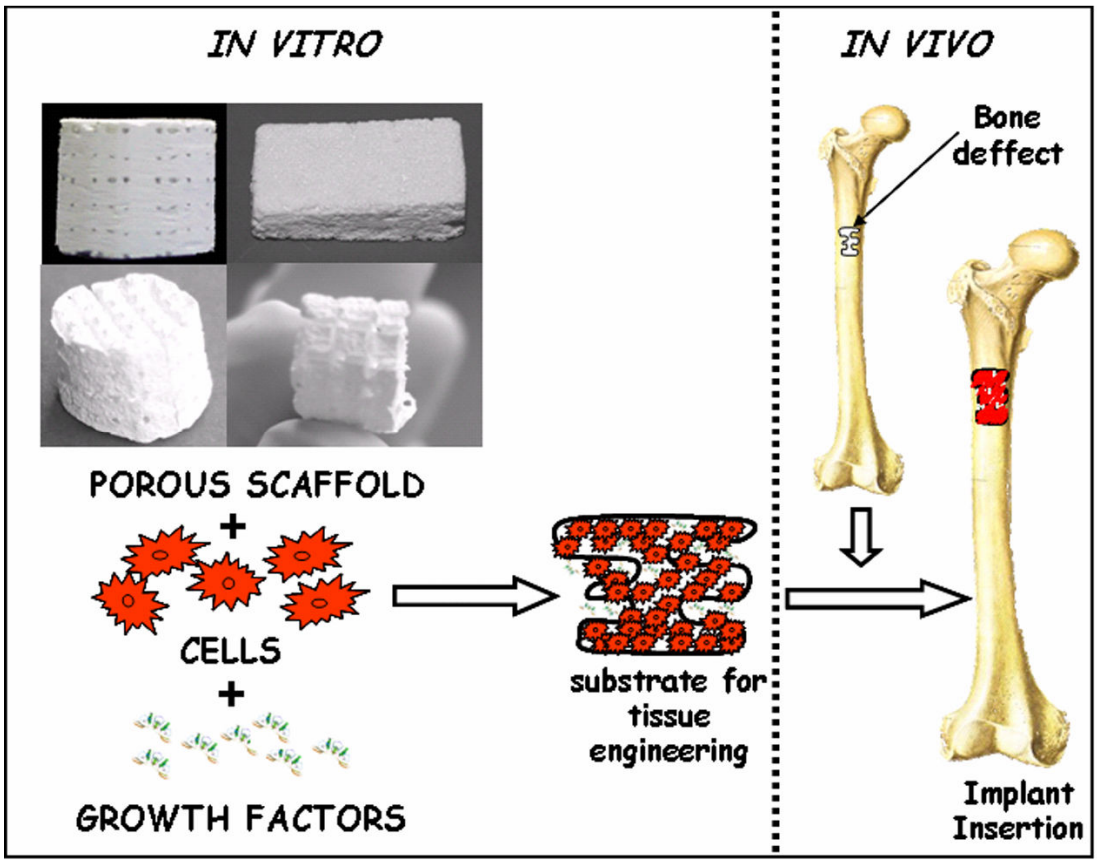

Figure 5. Schematic representation of different stages in a tissue engineering strategy.

Deville et al. [73] have fabricated freeze casting porous complex-shaped ceramic by directional freezing of the slurry. In this way, it is possible to force the particles in suspension to be rejected from the moving ice front and piled up between the growing columnar ice crystals. The microstructural features can be controlled by exploiting the physics of ice formation. Afterwards, the ice is sublimated by freeze drying in such a way that a CaP scaffold whose microstructure is a negative replica of the ice is produced. The width of the open interconnected macropores ranges between 20 and 100 μm in their smallest dimension and 50–500 μm in the largest one.

However, from the point of view of the requirements of an ideal scaffold, it is interesting to manufacture macroporous scaffolds with controlled and designed pore architecture. The gel-casting method allows conforming pieces with good homogeneity, minimized defects and high density of the ceramic matrix as well as to complex shapes and tailored geometry [74]. On the other hand, stereolithography is one of the main systems of the solid free form (SFF) fabrication technologies [75]. SFF it is a computer-controlled fabrication method that are based on the premise that a material in either powdered or

liquid form is solidified three-dimensional objects by forming successive cross-sectional laminae, one on top of another till the whole object is shaped. A homogenous suspension of BCP ceramic is poured on an epoxy resin negative previously designed by stereolithography. The obtained green pieces would be sintered to eliminate the epoxy resin leading the apropiated interconnected channels. The below methods, require firstly, the pirolyzation of the organic phases such as porogens, binders, dispersing agents, etc. and secondly, a sintering treatment of scaffolds in order to generate a macroporous system. Nevertheless, a thermal teatment step is not advisable because it would exponentially increase the particle size and the cristalinity of the BCP matrix and decreasing the bioreabsorption of the scaffold. For that issue, low shaping temperature methods have been developed. One example is the combination of this method with the stereolithography technique. The main objective of this method is to find a biodegradable and biocompatible binder of the BCP matrix, such as agarose hydrogel [76]. This combination consist of filled the moulds with the epoxy resin negative, previously mentioned, and afterwards, with a homogeneous suspension of HA/β-TCP/agarose. The epoxy resin negatives are removal by soaking the just prepared scaffolds in alkali solution. These pieces could be used as carriers of biotech products. Finally, the pieces are dried at room temperature and by freeze-drying leading to scaffolds with designed macropore architecture of about 300 to 460 μm.

Although the tailored fabrication of BCP based scaffolds provides excellent expectative, there are no many reported literature about the *in vivo* bone formation in designed and controlled architecture scaffolds using the SFF fabrication techniques and most of the BCP scaffolds tested are fabricated by classical techniques [77,78].

In an effort to produce clinically useful volumes of tissue engineering bone products, macroporous scaffolds obtained by conventional blocks fabrication (replica of porous sponges, production of gas bubbles, introduction of porogens etc.) have been assayed into bioreactor systems in the last years. As an example direct perfusion bioreactor systems are capable of giving rise to a interconnected hybrid structures and extracelular matrix into the BCP scaffolds with interconnected macroporosity of 388 μm [79]. Sendemir-Urkmer et al. [80] have developed a chitosan/BCP scaffolds with macroporosity of 100 μm aprox. where it was possible to enhances bone tissue development in a Boyden chamber. The hybrid macroporous BCP/bone cells has been recently evaluated *in vivo*, which means a decisive step to complete the bone tissue engineering strategy [81].

Osteogenesis and subsequent osteoconduction into interconnected BCP macropores of 200-800 μm was observed at 7 weeks of implantation.

Improving the biological response: Silicon doped BCPs.

Silicon plays and important role during the growth and development of bone and cartilage system. The earlier works carried out by Carlisle [82-84] reported the detection of silicon in vivo within the unmineralized osteoid region (active calcification regions) of the young bone of mice and rats. Silicon levels up to 0.5 wt% were observed in these areas, suggesting that Si has an important role in the bone mineralization process. Aqueous Si in the form of $Si(OH)_4$ induce the precipitation of HA in the presence of proteins that commonly inhibit its precipitation [85], healthy skeletal development is highly dependent on the dietary Si [86] and the collagen amount in bone and cartilage is significantly reduced in animals deprived of Si [87]. Therefore, Si is currently categorized as an essential trace element for metabolic processes associated with development of bone and connective tissues [86,88].

The appearance 1971 of SiO_2-based materials for bone grafting in the form of bioactive glasses, meant an outstanding advance in the field of bone repairing therapies [89,90]. However the silicon or silicate incorporation into the calcium phosphate (CaP) implants is relatively recent, with the synthesis of Si substituted HA and TCP [91-93]. Currently, it is widely accepted that Si substituted CaP exhibit an improved in vivo performance compared to non substituted CaP based implants. In this sense, Si-HA and Si-TCP based materials exhibit enhanced bone apposition, bone in-growth [94,95] and cell-mediated degradation in comparison to stoichiometric HA and TCP [96,97]. These biological events are closely related with the higher biomimetic apatite growth on their surfaces [98-101] as well as the improved solubility, associated to an increased adsorption and incorporation of biological moieties that are thought to serve as attachment sites for cells. From the point of view of materials science, the Si or silicates incorporation into HA or TCP materials leads to important changes in surface chemistry, crystalline strains and microstructural features, which can explains the enhanced reactivity and in vivo performance of these compounds [102-105].

Silicon substituted CaPs have been synthesised by different chemical methods, each of them exhibiting advantages and drawbacks inherent to the driving force that lead to the reaction. For instance, high temperatures solid state reactions have been employed to

obtain Si substituted HA, leading to highly crystalline structures that facilitate the study by diffraction techniques [106]. Other methods such as mechanochemical [107] or hydrothermal synthesis [108] allows to obtain Si substituted CaP with small grain sizes, high strains levels and, consequently more reactive. However, silicon BCP are mainly synthesised by wet chemical methods, by incorporating a silicon source in the reaction media. Silicon is commonly incorporated as an alcoxide, TEOS or TPOS, which is hydrolyzed in the aqueous media [109,110], or as a salt such as Si acetate suspended in the media [92]. In this cases, CO_2 is produced during the firing of the as precipitated product, and can be incorporated as carbonates into the HA or TCP structure. An alternative method is to incorporate silica nanoparticles during the precipitation of HA or amorphous calcium phosphate [109,111,112]. The final phase composition, *i.e* HA-TCP ratio is highly dependent on the Si added as well as the kind of silicon source used.

The most evident model of silicon or silicate incorporation into HA and TCP is that PO_4^{3-} is substituted by SiO_4^{4-}. The mechanism that compensates the extra negative charge will depend on the thermodynamic conditions. Therefore, during the synthesis of BCPs with Si stabilized α-TCP, the thermal treatment is determinant. Among the different proposed mechanisms, the literature collects Ca^{2+} and H^+ excess, as well as OH^- (in the case of HA) or O_2^- vacancies [92,93,95] and it is highly dependent on the water available in the reaction media. General formulae like $Ca_{10}(PO_4)_{6-x}(SiO_4)_xOH_{1-x}$ and $Ca_3(P_{1-x}Si_xO_{4-x/2})_2$ have been proposed for Si-HA and Si-αTCP, respectively [92,95,112,113], although the final BCPs products seems to be much more complex and often contains amorphous phases (ACP, amorphous silica) or calcium silicates [110,114]. Anyway, this substitution leads to BCPs with higher solubility, more crystalline strains, smaller grains sizes and higher number of triple junctions. All this changes added to an increase in negative surface charge promote the biomimetic processes that would justify the excellent in vivo behaviour of these bioceramics.

Silicon or silicates incorporation determine the thermal stability of CaP phases. As explaining above, the synthesis of HA/β-TCP biphasic materials are commonly carried out by heating CDH above 700ºC, whereas α-TCP is obtained when thermal treatments about 1125ºC are used. When SiO_2 is present during CDH heating, α-TCP HA biphasic materials can be obtained at temperatures as low as 700ºC. The α-TCP so obtained is denoted as silicon stabilized α-TCP and different combinations of biphasic calcium phosphates [109,111] or pure α-TCP [112] have been synthesised.

Although α-TCP and β-TCP have the same chemical composition, they differ by the crystal structure and solubility. These differences make α-TCP a more reactive and soluble phase in aqueous systems. Multiphase Si stabilized calcium phosphate mixtures are currently commercialized under the trade name Skelite™ with applications for filling of small bone defects in both microporous scaffolds or granulates. Single phase Si-HA is also commercialized for the same purposes with the trade name Actifuse™.

Summary and outlook

In this work we have reviewed different strategies for the synthesis and processing of BCPs, aimed to optimize bioceramic bone implants and for the fabrication of scaffolds for bone tissue engineering. Calcium phosphates exhibit an excellent biocompatibility and more especially, HA shows a high degree of osteoconductivity due to the bioactive bond formation with the bone tissue. Due to that, HA has been a bioceramic of reference in the field of calcium phosphate for medical applications, and subsequently improved by associating it to a more soluble component such as β-TCP. However, the development of bone implants has led to the appearance of third generation bioceramics, where bone regeneration is the main objective instead of bone substitution. BCP bioceramics, which were intended as osteoconductor materials in the past, stand for materials to fabricate osteoinductor implants in the near future. As it has been described in this review, the first steps has been given by fabricating BCP based scaffolds for bone tissue engineering, through the design of controlled 3D-porous structures, or increasing the biological activity through the incorporation of Si into the calcium phosphate (both HA and TCP) structures.

The control of the composition and hierarchical porous structure will allow in a near future to obtain scaffolds that can host osteoinductor biotech products (growth factors, hormones, etc.). This stage will mean a great advance towards the aim of high efficient bone regeneration.

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References

[1] F.H. Albee and H.F. Morrison: Ann. Surg. Vol. 71 (1920), p. 32.

[2] M. Jarcho: Den. Clin. N. Am. Vol. 30 (1986), p.25.

[3] J.N. Kent, J.H. Quinn, M.F. Zide, M.S. Block and M. Jarcho: J. Den. Res. Vol. 63 (1984), p. 325.

[4] J.N. Kent, J.H. Quinn, M.F. Zide, I.M. Finger, M. Jarcho and S.S. Rothstein: J. Amer. Dent. Assoc. Vol. 105 (1982), p. 993.

[5] M. Jarcho: Clin. Orthop. Related Res. Vol. 157 (1981), p. 259.

[6] S.B Dorozhkin and M. Epple: Angew. Chem. Int. Ed. Vol. 41 (2002), p. 3130.

[7] J. R. Hupp and S.J. McKenna: J. Oral Maxillofac. Surg. Vol. 46 (1988), p. 533.

[8] M. El Deeb and M. Roszkowski: J. Oral Maxillofac. Surg. Vol. 46 (1988), p. 33.

[9] B.V. Rejda, J.G.J Peelen and K. de Groot: J. Bioengn. Vol. 1 (1977), p. 93.

[10] G. Daculsi, LeGeros R.Z, E. Nery, K. Lynch and B. Kerebel. J. Biomed. Mater. Re. Vol. 23 (1988), p. 257.

[11] M. Vallet-Regí: J. Chem. Soc. Dalton Trans. (2001), p. 97.

[12] R. Ellinger, E.B. Nery and K.L. Lynch: Int. J. Periodont. Tertor. Dent. Vol. 3 (1986), p. 23.

[13] A. Takeishi, H. Hayashi, H. Kamatsubara, A. Yokoyama, M. Kohri, T. Kawasaki, K. Micki and T. Kohgo: J. Dent. Res., Vol. 68 (1989), p. 680.

[14] G. Daculsi, N. Passuti, S. Martin, C. Deudon, R.Z. LeGeros and S. Rather: J. Biomed. Mater. Res., Vol. 24 (1990), p. 379.

[15] C. Schopper, F. Ziya-Ghazvini, W. Goriwoda, D. Moser, F. Wanschitz, E. Spassova, G. Lagogiannis, A. Auterith and R. Ewers: J. Biomed Mater. Res. Part B: Appl. Biomater. Vol. 74B (2005), p. 458.

[16] E.B. Nery, K.L. Lynch and W.M. Hirthe: J. Periodontol. Vol. 63 (1975), p. 729.

[17] M. Trecant, J. Delecrin, J. Royer, E. Goyenvalle and G. Daculsi : Clin. Mater. Vol. 18 (1994), p. 233.

[18] A. Sendemir-Urkmez and R.D. Jamison : J. Biomed. Mater. Res. Vol. 81A (2007), p. 624.

[19] C.R. Yang, Y.J. Wang, X.F. Chen and N.R. Zhao. Mater. Lett. Vol. 59 (2005), p. 3635.

[20] M.I. Alam, I. Asahina, K. Ohmmaiuda and S. Enomoto: J. Biomed. Mater. Res. Vol. 54 (2000), p. 129.

[21] O. Gauthier, J. Guicheux, G.R. Grimandi, A. Faivre-Cahuvet and G. Daculsi: J. Biomed. Mater. Res. Vol. 40 (1998), p. 606.

[22] Groot K: Biomaterials Vol. 1 (1980), p. 47.

[23] M. Akao and H. Aoki, K. Kato: J. Mater. Sci. Vol. 16 (1981), p. 809.

[24] R. Z. LeGeros, in: Monographs in Oral Sciences, edited by H.M. Myers, volume 15 of calcium phosphates in oral biology and medicine, S. Karger Basel (1991).

[25] S.V. Dorozhkin: Key Eng. Mat. Vol. 192–195 (2001), p. 155.

[26] X. Yang and Z. Wang: J. Mater. Chem. Vol. 8 (1998), p. 2233.

[27] R. Ramachandra Rao, H. N. Roopa, and T. S. Kannan: J. Mater. Sci: Mater. Med. Vol. 8 (1997), p. 511.

[28] J.M. Bouler, R.Z. LeGeros, G. Daculsi: J. Biomed. Mater. Res. Vol. 51 (2000), p.680.

[29] E.I. Dorozhkina and S.V. Dorozhkin: Chem. Mater. Vol. 14 (2002), p. 4267.

[30] J.C. Elliot, in: Structure and chemistry of the apatites and other calcium orthophosphates, Studies in inorganic chemistry, Elsevier (1994).

[31] K. S. TenHuisen and P. W. Brown: Biomaterials Vol. 19 (1998), p. 2209.

[32] M. Andrés-Vergés, C. Fernández-González and M. Martínez-Gallego: Eur. Ceram. Soc. Vol. 18 (1998), p. 1245.

[33] S. Raynaud, E. Champion, D. Bernache-Assollant and Thomas P: Biomaterials Vol. 23 (2002), p. 1065.

[34] M. Vallet-Regí, L.M. Rodríguez-Lorenzo and AJ Salinas: Solid State Ionics Vol. 101-103 (1997), p. 183.

[35] N. Kivrak and A. Cuney Tas. J. Am. Ceram. Soc .Vol. 82 (1998), p.2245.

[36] S. H. Kwon, Y.K. Jun, S. H. Hong and H. E. Kim: J. Eur. Ceram. Soc. Vol. 23 (2003), p. 1039.

[37] A. Cunneyt Tas: J. Eur. Ceram. Soc. Vol. 20 (2000), p. 2389.

[38] I. Manjubala and M. Sivakimar: Mater. Chem. Phys. Vol. 71 (2001) p. 272.

[39] F.H. Lin, C.J. Liao, K.S. Chen, J.S. Sun, C.Y. Lin: J. Biomed. Mater. Res. Vol. 51 (2000), p. 157.

[40] N. Bhaskar, J. M. Brady, L. Getter, M. F. Grower and T. Driskell, J. Oral Surg. Vol. 32 (1971), p. 336.

[41] R.Z. LeGeros: Adv. Dent. Res. Vol. 2 (1988), p. 164.

[42] G. Daculsi, RZ LeGeros E. Nery , Lynch K. , Kerebel B. J Biomed Mat Res Vol. 23 (1989), p. 883.

[43] R.Z. LeGeros and G. Daculsi, In: Handbook of Bioactive Ceramics. In vivo transformation of biphasic calcium phosphate ceramics: ultrastructural and physico-chemical characterizations, edited by Yamamuro N, Hench L, Wilson-Hench, J, volume 2 of Calcium Phosphate Ceramics, CRC Press Boca Raton (1990).

[44] H. Nakahara, V.M. Goldberg and A.I. Caplan. Clin. Orthop. Vol. 276 (1992), p. 291.

[45] J. Goshima, V.M. Goldberg and A.I. Caplan: Biomaterials Vol. 12 (1991), p. 253.

[46] P. Frayssinet, J. L. Trouillet, N. Rouquet, E. Azimus, and A. Autefage: Biomaterials Vol. 14 (1993), p. 423.

[47] J. Delecrin, S. Takahashi, F. Gouin and N. Passuti: Spine Vol. 25 (2000), p. 563.

[48] G. Daculsi, E. Goyenvalle and E. Aguado: Bioceramics Vol. 12 (1999), p. 287.

[49] R. Cavagna, G. Daculsi and J.M. Bouler: J. Long. Term. Eff. Med. Implants Vol. 9 (1999), p. 403.

[50] A.O. Ransford, T. Morley, M.A. Edgar, P. Webb, N. Passuti, D. Chopin, C. Morin, F. Michel, C. Garin and D. Pries. J. Bone Joint. Surg. Br. Vol. 80 (1998) p.13.

[51] N. Passuti, G. Daculsi, J. M. Rogez, S. Martin, and J. V. Bainvel: Clin. Orthop. Vol. 248 (1989), p. 169.

[52] O. Gauthier, E. Goyenvalle, J.M. Bouler, J. Guicheux, P. Pilet and P. Weiss G Daculsi: J. Mater. Sci.: Mater. Med. Vol. 12 (2001), p. 385.

[53] O. Gauthier, J.M. Bouler, E. Aguado, R.Z. LeGeros, P. Pilet, G. Daculsi: J. Mater. Sci.: Mater. Med. Vol. 10 (1999), p. 199.

[54] J. Toquet, R. Rohanizadeh, J. Guicheux, S. Couillaud, N. Passuti, G. Daculsi and D. Heymann: J. Biomed. Mater. Res. Vol. 44 (1999), p. 98.

[55] O. Gauthier, J.M. Bouler, E. Aguado, P. Pilet and G. Daculsi: Biomaterials Vol. 19 (1998), p. 133.

[56] K. Anselme: Biomaterials Vol. 21 (2000), p. 667.

[57] S. Yamada, D. Heyman, J.M.Bouler and G. Daculsi: Biomaterials Vol. 18 (1997) p. 1037.

[58] S. Sánchez-Salcedo, I. Izquierdo-Barba, D. Arcos and M. Vallet-Regí: Tissue Engin. Vol 12 (2) (2006) p. 279.

[59] J.E. Dennis, S.E. Haynesworth, R.G. Young and A.I. Caplan: Cell. Transpl. Vol. 1 (1991), p. 23.

[60] H. Ohgushi, V.M. Goldberg and A.I: Caplan. J. Orthop. Res. Vol. 7 (1989), p. 568.

[61] P. Ducheyne and Q. Qiu: Biomaterials Vol. 20 (1999), p. 2287.

[62] M. Hashimoto Uohima, I. Ishidawa, A. Kinoshita, H.T. Weng and S. Oda: Int. J. Periodont. Rest. Dent. Vol. 15 (1995), p.204.

[63] A.Piattelli, A.Scarano, C. Mangano: Biomaterials Vol. 17 (18) (1996), p. 1767.

[64] G. Daculsi, P. Weiss, J.M. Bouler, O. Gauthier, F. Millot, E. Aguado: Bone Vol. 25 (2) (1999), p. 59S.

[65] O. Gauthier, J.M. Bouler, P. Weiss, J. Bosco, G. Daculsi, and E. Aguado: J. Biomed. Mater. Res. Vol. 47 (1999), p. 28.

[66] O. Malard, J.M. Bouler, J. Guicheux, D. Heymann, P. Pilet, C. Coquard, and G. Daculsi: J. Biomed. Mater. Res. Vol. 46 (1999), p. 103.

[67] J.M. Karp, P.D. Dalton and M.S. Shoichet: MRS Bull. Vol. 28 (2003), p. 301.

[68] P.S. Eggli, W. Muller and CRK. Schenk: Clin. Orthop. Vol. 232 (1988), p. 127.

[69] M. Doblaré, J.M. García and M.J. Gómez: Eng. Fract. Mech. Vol. 71 (2004), 1809.

[70] J.J. Klawitter and S.F. Hulbert: J. Biomed. Mater. Res. Sym. Vol. 2 (1971), p. 161.

[71] J.X. Lu, B. Flautre, K. Anselme and P. Hardouin: J. Mater. Sci.: Mater. Med. Vol. 10 (1999), p. 111.

[72] S. Sánchez-Salcedo, J. Werner and M. Vallet-Regí: Acta Biomaterialia (submitted)

[73] S. Deville, E. Saiz, A. P. Tomsia: Biomaterials Vol. 27 (2006), p. 5480.

[74] S. Padilla, S. Sánchez-Salcedo and M. Vallet-Regi: J. Biomed. Mater. Res. Vol. 81A (2007), p. 224.

[75] C. Hull, U.S. Patent 4,575,330. (1986)

[76] S. Sánchez-Salcedo, A. Nieto and M. Vallet-Regí: Chem. Engin. J. On line 2007.

[77] C.T. Buckley and K.U. O'Kelly, in: Bio-Mechanical Engineering, edited by P.J. Prendergast and P.E. McHugh, (2004).

[78] D.W. Hutmacher: J. Cell. Mol. Med. Vol. 11 (4) (2007), p. 654.

[79] F.W. Janssen, I. Hofland, A. van Oorschot, J. Oostra and H. Peters, C.A. van Blitterswijk: J. Biomed. Mater. Res. 79A (2006), p. 338.

[80] A. Sendemir-Urkmez and R D. Jamison: J. Biomed. Mater. Res. 81A (2007), p. 624.

[81] M.C. Kruyt, J.A Wouter, F. Dhert, C.O. Clemens and A. van Blitterswijk: Biomaterials 28 (2007), p. 1798.

[82] E. Carlisle: Science 167 (1970), p. 279.

[83] E. Carlisle: Science 178 (1972), p. 619.

[84] E. Carlisle: J Nutr. 110 (1979), p.1046.

[85] J. Damen and J. Ten Cate: J. Dent. Res. 71 (1992), p.453.

[86] E. Carlisle: Nutr Rev. 40 (1982), p.193.

[87] C. Seaborn and F. Nielson: Biol Trace Elem Res 89 (2002), p. 251.

[88] E. Carlisle: Sci Total Environ 73 (1988) p. 95.

[89] L. L. Hench, R. J. Splinter, T. K. Greenlee and W. C. Allen: J. Biomed. Mater. Res. 2 (1971), p.117.

[90] M. Vallet-Regi, A.J. Salinas and D. Arcos: J. Mater Sci: Mater Med 17 (2006), p. 1011.

[91] A.M. Pietak, J.W. Reid, M.J. Stott and M. Sayer: Biomaterials 28 (2007), p. 4023.

[92] I. Gibson, S. Best and W. Bonfield: J. Biomed. Mater. Res. 44 (1999) p. 422.

[93] M. Vallet-Regí and D. Arcos: J. Mater. Chem 15 (2005) p.1509.

[94] M. Mastrogiacoma, A. Corsi, E. Francisco, M. Di Comite, F. Monetti and S. Scaglione: Tissue Eng. 12, (2006), p. 1261.

[95] S. Langstaff, M. Sayer, T. Smith, S. Pugh, S. Hesp and W. Thompson: Biomaterials 22 (2001), p.135.

[96] C. Botelho, R. Brooks, G. Spence, I. McFarlane, M. Lopes, S. Best, J.D. Santos, N. Rushton and W. Bonfield: J. Biomed. Mater. Res A 78 (2006), p.709.

[97] N. Patel, S. Best, W. Bonfield, I. Gibson, K. Hing, E. Damien, et al.: J. Mater. Sci. Mater Med, 13 (2002), p.1199.

[98] D. Arcos, S. Sánchez-Salcedo, I. Izquierdo-Barba, L. Ruiz, J. Gónzalez-Calbet and M. Vallet-Regí: J. Biomed. Mater. Res. A, 78 (2006), 78 p.762.

[99] F. Balas, J. Pérez-Pariente and M. Vallet-Regí: J. Biomed. Mater. Res. A 66 (2003), p. 364.

[100] X. Li, H. Yasuda and Y. Umakoshi: J. Mater. Sci Mater. Med 17 (2006), p.573.

[101] M.Vallet-Regí: Dalton Trans. (2006), p. 5211.

[102] M. Sayer, A. Stratilatov, J. Reid, L. Calderin, M. Stott, X. Yin, M. McKenzie, T.J.N.Smith, J.A. Hendry and S.D. Langstaff: Biomaterials 24 (2003), p.369.

[103] A. Porter: Micron 37, (2006), p.681.

[104] A. Pietak and M. Sayer: Biomaterials 24 (2005), p.3819.

[105] C. Botelho, M. Lopez, I. Gibson, S. Best, J. Santos: J. Mater Sci Mater Med 13 (2002), p.1123.

[106] D. Arcos, J. Rodríguez-Carvajal and M. Vallet-Regí: Solid State Sci 6 (2004), p. 987.

[107] C. Mochales, M. El Briah-BenAbdeslam, M. Ginebra, P. Boudeville and J. Planell: Key Eng Mater 254-256 (2004), p.107.

[108] X. Tang, X. Xiao and R. Liu: Mater Lett 59, (2005), p. 3841.

[109] S. Langstaff, M. Sayer, T. Smith, S. Pugh, S. Hesp and W. Thompsom: Biomaterials 20 (1999), p.1727.

[110] D. Arcos, J. Rodríguez-Carvajal and M. Vallet-Regí: Chem. Mater. 16 (2004), p. 2300.

[111] J. Reid, A. Pietak, M. Sayer, D. Dunfield and T. Smith: Biomaterials 26 (2005), p. 2887.

[112] J. Reid, L. Tuck, M. Sayer, K. Fargo and J. Hendry: Biomaterials 27 (2005), p.2916.

[113] T. Leventouri, C.Bunaciu and V. Perdikatsis: Biomaterials 24 (2003), p. 4205.

[114] A. Tuys: J. Aust Ceram Soc 29 (1993), p.71.