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Silicon calcium phosphate ceramic as novel biomaterial to simulate the bone regenerative properties of autologous bone

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Abstract: This study was conducted to develop novel ceramic bone substitute that resembles the autologous bone behavior when used as graft material. Solid-state reaction at 1100°C was performed to synthesize β-tricalcium phosphate (β-TCP) and biphasic calcium phosphate (BCP). The ceramics were further analyzed to characterize phase composition, microstructural properties, cytocompatability and then challenged to regenerate critical bone defects in the parietal bone of rabbits. X-ray diffraction analysis confirmed the production of β-TCP and indicated the synthesis of novel BCP composed of β-TCP and silicocarnotite (calcium phosphate silicate mineral). The cytocompatibility test with human osteoblast cell line revealed enhanced cell proliferation on the BCP ceramic. The novel BCP induced the filling of about 73% of the bone defect with a newly formed bone tissue and an almost complete degradation after 12 weeks of healing. This novel ceramic resembles the autologous bone properties of complete degradation and efficient enhancement of bone formation, making it promising as bone graft material. © 2014 Wiley Periodicals, Inc. J Biomed Mater Res Part A: 00A:000–000, 2014.

Key Words: biphasic calcium phosphate, silicon doped ceramic, bone substitution, tricalcium phosphate, bone graft

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INTRODUCTION

Bone tissue is susceptible to suffer injury caused by endogenous factor (cysts, tumors, or congenital deformities) or by exogenous factors (injuries from traffic accidents or warfare) that leads to a loss of tissue substance. The treatment of such osseous tissue loss in many occasions requires the use of bone regeneration techniques. Over the last several decades, high demand for devices used in bone regeneration has encouraged the development of new biomaterials and less invasive surgery techniques with minor morbidity.[1–4] Although a lot of advances have been attained, scientists have not yet achieved a biomaterial that can be as efficient or superior to autologous bone to regenerate bone defects.[5,6] This is evident by the fact that until now autologous bone is still considered the gold standard in bone regeneration.

Clinically the main bone substitutes in use are calcium phosphates and these are principally selected from the group of hydroxyapatite (HA), alpha and beta-tricalcium phosphate (α/β-TCP) known as tricalcium phosphate (TCP) or a combination of both ceramics [biphasic calcium phosphate (BCP)]. Stoichiometric HA [$(Ca_{10}(PO_4)_6(OH)_2)$] has a low degradation rate that may cause implant loss and poor handling characteristics.[7] On the other hand, β-TCP [β-$Ca_3(PO_4)_2$] has higher osteotransductive properties but a lower strength and poor mechanical properties. To improve their properties and *in vivo* behavior, this has led to the development of different BCPs mixtures with variable quantities of HA and β-TCP. To improve the regenerative potential of these biomaterials, scientists have followed two main strategies: (i) the use of growth factors (recombinant or autologous)[8] and (ii) ionic substitution as some metallic ions have documented biological effect on bone remodelling. In fact the mineral phase of the bone contains ions other than calcium and phosphate such as carbonate, strontium, sodium, potassium, barium, and silicon. These ions have been shown to determine the behavior of calcium phosphate-based biomaterials by modifying their solubility, morphology and cellular response.[9–12]

Silicon (Si) is the most common ion researched to achieve such a task since Carlisle[13] in 1970 showed the development of skeletal alterations in animals fed with a diet low in Si. Furthermore, it is known that Si plays a key role in the mineralization process around dental implants,

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having a stimulatory effect on such process with direct influence over osteoblast proliferation.[14,15] Increasing evidence is now available about the improved biological performance of Si-substituted calcium phosphate ceramics.[16–18] The bioactivity enhancement of Si-substituted calcium phosphates can be attributed to a number of factors that act synergistically. Si-substitution facilitates the precipitation of biological HA on implant surfaces[19–21] which promotes protein adsorption and osteoblast attachment and proliferation. This is added to the direct effect of Si either released to the extracellular matrix or present in the implant surface on osteoblasts, osteocalsts, and collagen synthesis.[22–27]

Furthermore in vivo implantation of silicon-substituted hydroxyapatite (Si-HA) granules in rabbits resulted in an increase of 14.5% bone in-growth compared to un-substituted HA. The formation of collagen fibrils on implant surface was observed after 6 weeks for Si-HA compared to the 12 weeks for un-substituted HA.[28] A silicon content of 0.8 wt% in porous HA scaffold resulted in better response in terms of bone apposition, in-growth and adaptive remodelling.[18] Calcium-deficient HA cement prepared with 1%-Si-α-TCP was implanted in rabbits and shown to have a positive effect on osteoclastic and osteoblastic activity as well as osteointegration.[17] Silicon-substituted tricalcium phosphate based scaffolds showed good degradability and complete resorption and replacement by new bone tissue after 2 years.[29] However, there is no significant evidence that appeals for the enhancement of *in vivo* resorption of Si-HA ceramics.[30]

In our attempt to develop a biomaterial that resembles autologous bone properties of complete biodegradability and high capacity to stimulate bone regeneration we developed a novel biphasic biomaterial composed of silicon-doped beta-tricalcium phosphate and silicocarnotite (similar to HA where one phosphate group is substituted by a Si group). In this article we are presenting the properties of this biomaterial from the synthesis to the in vitro and in vivo performance to regenerate critical bone defects.

MATERIALS AND METHODS

Powder synthesis

β-TCP was obtained by solid state reaction of stoichiometric amounts of calcium carbonate (CC; Sigma–Aldrich) and dicalcium phosphate dihydrate (DCPD; Sigma–Aldrich) at 1100°C for 14 h. The novel biphasic ceramic was prepared by substituting DCPD with fumed SiO_2 (Sigma–Aldrich) to obtain a [Si/(Si+P)] ratio of 80% while keeping the molar [Ca/(P+Si)] ratio constant at 1.5. The amount of reagents was calculated on the assumption that SiO_4^{4-} would substitute PO_4^{3-} in β-TCP. The sintered ceramics were crushed and sieved to select ceramic particles with a diameter between 0.5–0.8 mm.

Powder X-ray diffraction patterns were recorded on an X'Pert Philips diffractometer using Cu Ka radiation (45 KV, 40 mA). X-ray diffraction was recorded at an interval of $2\theta = 3°–60°$ with a step size of 0.02° and a counting time of 1 s per step. The mineral composition of the ceramic powder was confirmed by employing JCPDS database for β-TCP, silicocarnotite and HA (pdf-ref 55–898, pdf-ref 40–393, and pdf-ref 73–1731, respectively). The ceramics microstructure was examined using a JSM 6400 scanning electron microscope (SEM). The micrographs obtained by SEM were processed using the Image J software to measure the major diameter of the ceramic grains and the data was expressed as mean ± standard deviation (SD). The specific surface area was measured by the Brunauer Emmet–Teller (BET, GEMINI, Micromeritics, USA) method, while the porosity and pore size distribution were determined by high pressure mercury porosimetry (Micromeritics 9420, UK).

In vitro test

The proliferation and viability of the human osteoblast-like cell line MG-63 (ATCC no. CRL-1427, Rockville, MD) grown in Dulbecco's Modified Eagle's Medium (DMEM, Invitrogen Life Technologies, Karlsruhe, Germany) containing 10% fetal calf serum, 1% penicillin, and streptomycin (Invitrogen Life Technologies) was determined by means of cell counting and WST-1 test (Roche Diagnostics, Mannheim, Germany). For this purpose 0.07 g of β-TCP and BCP were placed in triplicate into a 24-well plates, so that the bottom of each well was completely covered by the material. As the powder would float during pipetting the cell suspension, 300 μL of 1% agar solution in PBS was added and left to gelate to prevent the floating of the powder. Once the agar is gelled cells were seeded onto the ceramics at an initial density of 25,000 cells per well into 500 μL of DMEM. Cell culture wells coated with only 1% agar served as a control. Cell proliferation and cell viability was determined after 3, 5, 7, and 10 days of culture.

Cell viability was determined using cell proliferation reagent WST-1. Cell line MG 63 was incubated for 30 min with WST reagent 1:100 in DMEM at 37°C and the supernatant was quantified in a Tecan spectrafluor plus photometer (Tecan, Crailsheim, Germany). Cell proliferation was measured by electronic cell counting using a CASY 1 TTC cell analyzer (Schärfe System, Reutlingen, Germany). For this purpose, cells were detached by incubation with Accutase (PAA, Cölbe, Germany) after two washes in PBS. The reaction was stopped by adding equal amounts of DMEM. After dilution 1:100 of the cell suspension in 10 mL Isoton III (Beckman Coulter, Krefeld) cells were counted and the number was calculated automatically by the Casy-stat software (Schärfe System, Reutlingen, Germany). For each analysis, the samples were examined in triplicate and the average and standard deviation were calculated. Results were expressed as mean ± SD. Statistical software Origin 8 (OriginLab Corporation, Northampton, USA) was used to analyze the data by two-Way ANOVA where differences between treatment groups were evaluated using Tukey *post hoc* testing. All results were considered to be significant at a significance level of ($p < 0.05$).

Ion release

Ca^{2+}, SiO_4^{4-}, and PO_4^{3-} release from β-TCP and BCP ceramics have been monitored for 7 days by their ageing in DMEM. The ageing medium was renewed every 24 h and the ions concentration was determined using inductively coupled plasma optical emission spectrometry (ICP-OES)

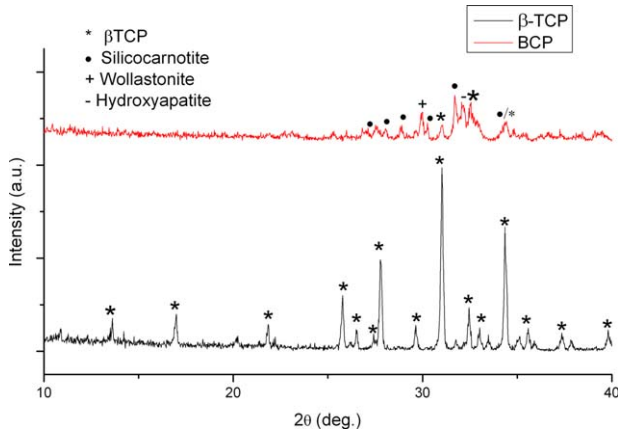

FIGURE 1. X-ray diffraction patterns of β-TCP (lower panel) and BCP (upper panel) ceramics. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

against standard solutions of Ca^{2+}, SiO_4^{4-}, and PO_4^{3-} obtained from Merck.

In vivo test

Prior to beginning the in vivo animal study, the protocol was approved by the ethical committee for animal experiments of the Rey Juan Carlos University (URJC). Eight healthy 6-month old, female New Zealand rabbits weighing between 3.9 and 4.4 kg were used. Proper care and maintenance of the animals were followed throughout this study [Animals Act, 1986 (86/609/EEC)] in order to minimize pain and discomfort. Materials were tested in vivo in granulated form with a size between 0.5 and 0.8 mm in diameter. Sample preparation was performed under aseptic conditions and samples were sterilized in an autoclave (Medioclave, JP Selecta, Barcelona, Spain) during 24 min at 121°C before implantation into the calvaria of rabbits. Surgery was performed on the eight rabbits, and anaesthesia was induced with an intramuscular injection of ketamine (35 mg kg^{-1}, Imalgene 1000®, Merial) and xylazine (5 mg kg^{-1}, Rompun®, Bayer).

The study was conducted in the rabbit's calvaria creating critical-size defects of 10 mm in diameter. Animals were shaved and the surgical site was disinfected with podivone iodine solution. Parietal bone was exposed after the practising of a 5 cm sagittal incision at the midline and elevating the periosteum. A stainless-steel trephine bur (10 mm in diameter) was employed to create three circumferential defects without invading the midline of the calvaria and the defects were then randomly filled with the ceramic granules. The periosteom was closed with a 3/0 resorbable suture (Dexon II® Davis & Geck, UK.) and the skin with a 4/0 silk suture (Apositos Sanitarios Aragoneses, Spain). Antibiotics (Terramicina, Pfizer, Spain) and analgesics (Buprenorphine, Schering-Plough, UK) were administrated during 3 days to prevent postsurgical infection and to control pain.

TABLE I. Average Pore Diameter, Porosity, and the Specific Surface Area of β-TCP and BCP Ceramics

Material	Avarege Pore Diameter (4V/A) (μm)	Hg-Porosity (%)	SSA BET ($m^2\ g^{-1}$)
β-TCP	7.5	77	0.816 ± 0.007
BCP	2.8	78	1.59 ± 0.01

TABLE II. The Fraction of Micropores (<10 μm), Mesopores (10–100 μm) and Macropores (>100 μm) for β-TCP and BCP Ceramics

Material	<10 μm (%)	10–100 μm (%)	>100 μm (%)
β-TCP	29	41	30
BCP	31	25	44

After animal sacrifice at 8 and 12 weeks to analyze the behavior of the bone regeneration materials, bone specimens were fixed in 10% buffered formaldehyde solution with a pH of 7.0. Specimens for histological/histomorphometric analysis were dehydrated in ascending concentrations of ethanol before they were embedded in polymerization resin (Technovit, Leica Microsystems, Germany). Sections 5- to 30-μm thick, were prepared and stained with methylene blue, toluidine blue and basic fuchsine. Histomorphometrical analysis was carried out using a light microscope (JVC TK C1380 Victor Co, Japan) coupled to an image analysis system IAS 2000 (Delta Sistemi, Italy) to quantify the amount of newly formed bone tissue, residual graft material and connective tissue within the grafted area. The amount of newly formed bone was expressed as a percentage of the total defect area. Variation in response between samples was analyzed statistically by using nonparametric Mann–Whitney test. $p < 0.05$ was considered to be statistically significant.

RESULTS

Materials characterization

Figure 1 shows the X-ray diffraction patterns of the newly synthesized BCP ceramic at a molar ratio of [Si/(Si+P)] 80%. The presence of new peaks in the diffraction patterns of the modified ceramic (Fig. 1) indicates the formation of a new crystalline phase known as silicocarnotite, which is a calcium phosphate silicate mineral. Other calcium silicate phase was identified as wollastonite. Furthermore a new peak was identified as HA. BCP sample showed a more amorphous structure due to the silicon incorporation.

Changes in the grains morphology were evident, the β-TCP grains are more round-like and the boundaries are more pronounced in comparison to the grains of BCP which acquired a more plate-like morphology. The average grains size was 1.72 ± 0.30 μm for β-TCP and 1.1 ± 0.2 μm for BCP. The high-pressure Hg porosimetry showed similar porosities for both ceramics, however the average pore size and the pore size distribution into micro-, meso-, and macro-pores showed significant differences between β-TCP and BCP (see Table I). β-TCP and BCP ceramics had a porosity of 77 and 78% with an average pore diameter of 7.5 and 2.8 μm, respectively. The distribution of pore size for both ceramics into micro-, meso-, and macro-pores is indicated in Table II. Compared to β-TCP ceramic, BCP ceramic

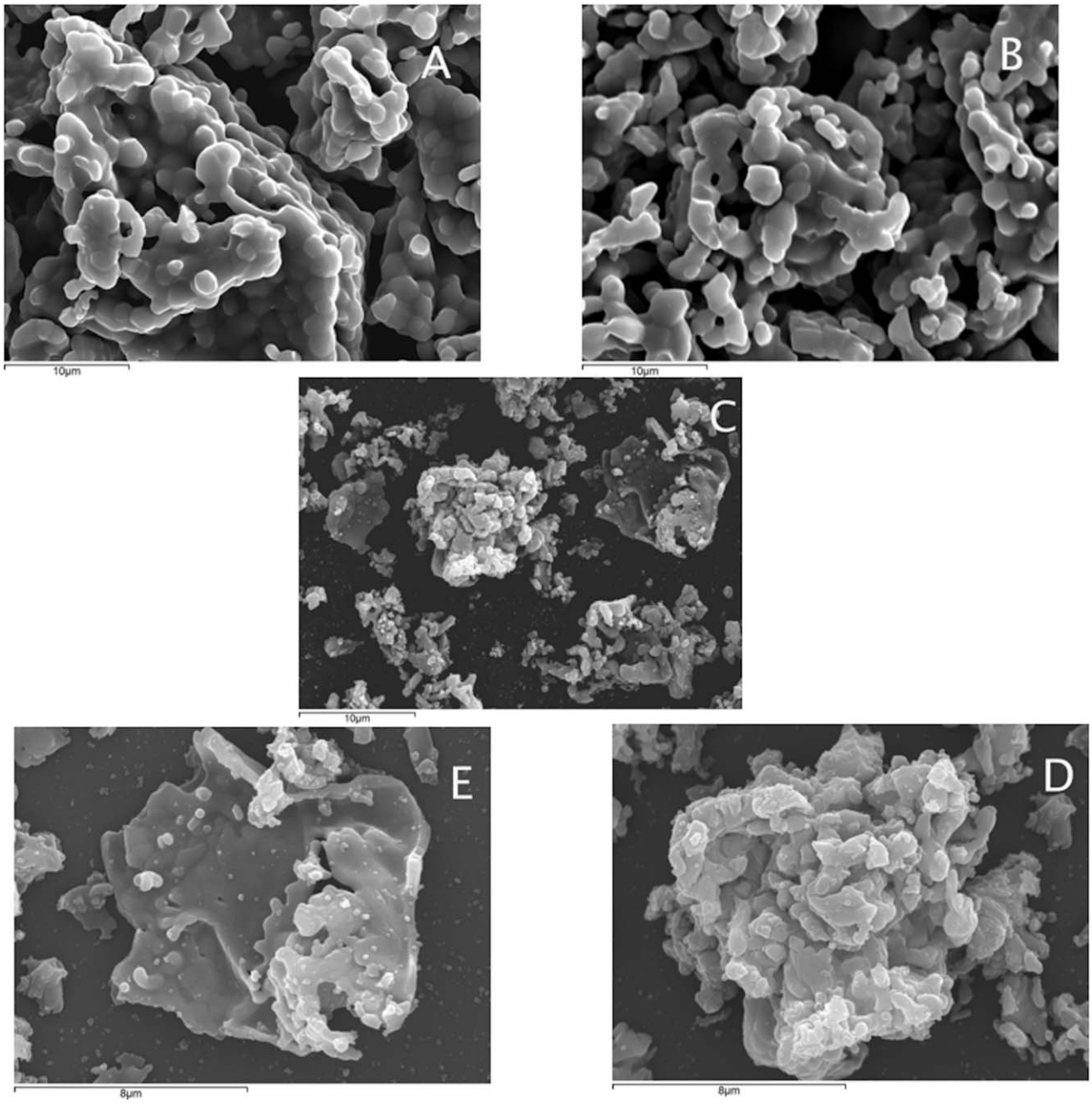

FIGURE 2. SEM images of the undoped β-TCP ceramic (A and B) and BCP (C,D and E).

had similar micro-pores (31 vs. 29%), lower the meso-pores fraction (25 vs. 41%) and increases by almost 50% the macro-pores population. Thus silicon ions have shifted the predominant pore type from meso-pores in β-TCP to the macropores in Si-modified ceramic. BET specific surface area (SSA) analysis shows that Si ions have increased significantly the SSA of BCP to 1.58 $m^2\ g^{-1}$ compared to almost 0.8 $m^2\ g^{-1}$ for the β-TCP ceramics.

In vitro results

The use of MG 63 cell line was able to detect differences in the capability and the dynamic of cell proliferation on the different ceramics. Comparing the results to the β-TCP, the BCP ceramic was able to enhance the cell proliferation as indicated in Figure 3. Although there were no significant differences between both ceramics at days 3, 5, and 7, BCP ceramic resulted in significantly higher cell proliferation after 10 days of culture. ANOVA statistical analysis indicated that both the material type and culture time are factors that affect significantly cellular proliferation, and the interaction between them is statistically significant.

The evaluation of cells with WST-1 reagent showed similar results in that silicon doping of the ceramic was able to induce differences compared to results of the β-TCP

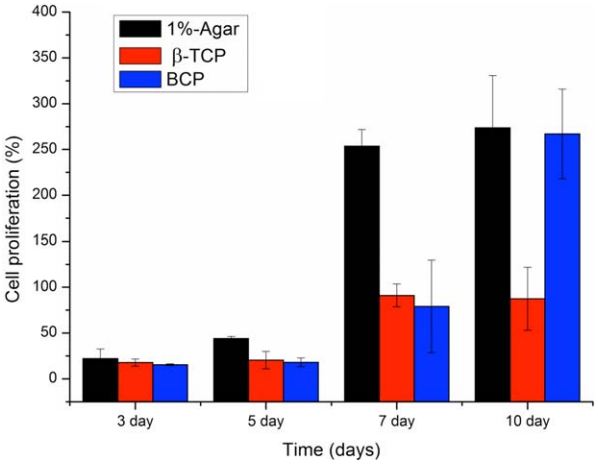

FIGURE 3. MG 63 cell proliferation assays at 10 days of seeding. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

(see Fig. 4). ANOVA statistical analysis indicates that type of material and culture time factors affect significantly the cellular activity as well as the interaction between both factors.

Ion release from ceramics

The Ca^{2+} release from β-TCP was characterized by an initial delivery of 70 ppm that decreased to 50 ppm at day 3 and then continuously increased to 80 ppm at day 7 [Fig. 5(A)]. The PO_4^{3-} release started with an initial burst of 22 ppm and then settled at about 14 ppm. Meanwhile BCP initially released Ca^{2+} at higher concentration (90 ppm), which later was settled at a lower concentration of 60 ppm [Fig. 5(B)]. The phosphate ions release from BCP was significantly lower than the β-TCP ceramic and had a maximum value of 2 ppm after the first day of incubation. Interestingly the BCP delivered SiO_4^{4-} at a constant concentration of 170 ppm throughout the observation period [Fig. 5(C)].

In vivo results

The animal's recovery after the surgery was uneventful. Differences between experimental groups were observed upon macroscopical examination of the samples after animal sacrifice. Unlike β-TCP, the site treated with the BCP showed almost complete graft resorption and its replacement by newly formed bone (Fig. 6). Microscopical analysis of the processed bone samples showed the absence of inflammatory infiltrates or an immunological reaction to the biomaterials. The bone formation in both groups started from the defect boundaries and continued thereafter to the center of the defect. The detailed finding from the histological study is reported in two parts: the first part describes the findings at intermediate healing time (8 weeks) and the second part describes the findings at late healing time (12 weeks).

Intermediate healing period (8 weeks)

The defects treated with β-TCP ceramic showed the presence of residual graft particles and the formation of new bone tissue [Fig. 7(A)]. A moderate presence of multinucleated giant cells could also be observed. In the histological sections at 5 μm, a large quantity of fibrous tissue with hypercellularity was observed, with no signs of inflammatory process, with the presence of multinucleated giant cells and with abundant mobilization of histiocytes. In some areas new bone formation was recognized as well as the presence of adipose tissue [Fig. 7(A,B)].

The BCP-treated defects showed an absence of inflammatory response and the presence of fibrous tissue. More new bone formation was reached in this group compared to β-TCP defects. Large quantities of osteoid and osteoblasts were observed inside the newly formed bone tissue. Also new blood vessels and several osseous bridges between bone trabeculaes could be observed in the BCP defect [Fig. 7(C,D)].

Late healing time (12 weeks)

After 12 weeks in β-TCP defects was evident the presence of more bone tissue formation compared with the samples harvested after 8 weeks of healing. Indeed a moderate quantity of newly bone formed surrounded by a border of osteoblasts and osteocytes incorporated to the mature bone tissue can be observed [Fig. 8(A)]. However fibrous tissue was predominant over new bone [Fig. 8(B)]. A decrease in the presence of residual graft material could be also observed.

While BCP ceramic induced the predominant presence of mature bone tissue over fibrous tissue, with coarse trabeculae and an important quantity of immature bone [Fig. 8(C)]. The presence of bone bridges flowing towards the center and the remnant material phagocytosed by giant cells could be observed [Fig. 8(D)]. Fusion of bone trabeculae and abundant peripheral osteoid could be recognized with the presence of osteoblasts and osteocytes. Interestingly, no remnants of biomaterial nor zones of macrophage accumulation could be observed [Fig. 8(C,D)].

Histomorphometric analysis

The newly formed bone (NB), remanent graft material and fibrous tissue were quantified and presented in

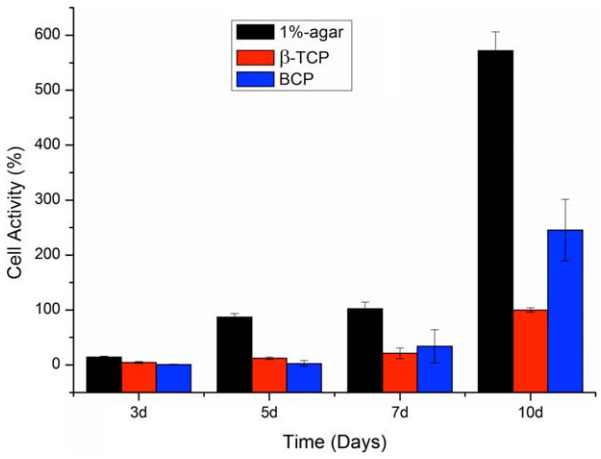

FIGURE 4. MG 63 cell activity assays at 10 days of seeding. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

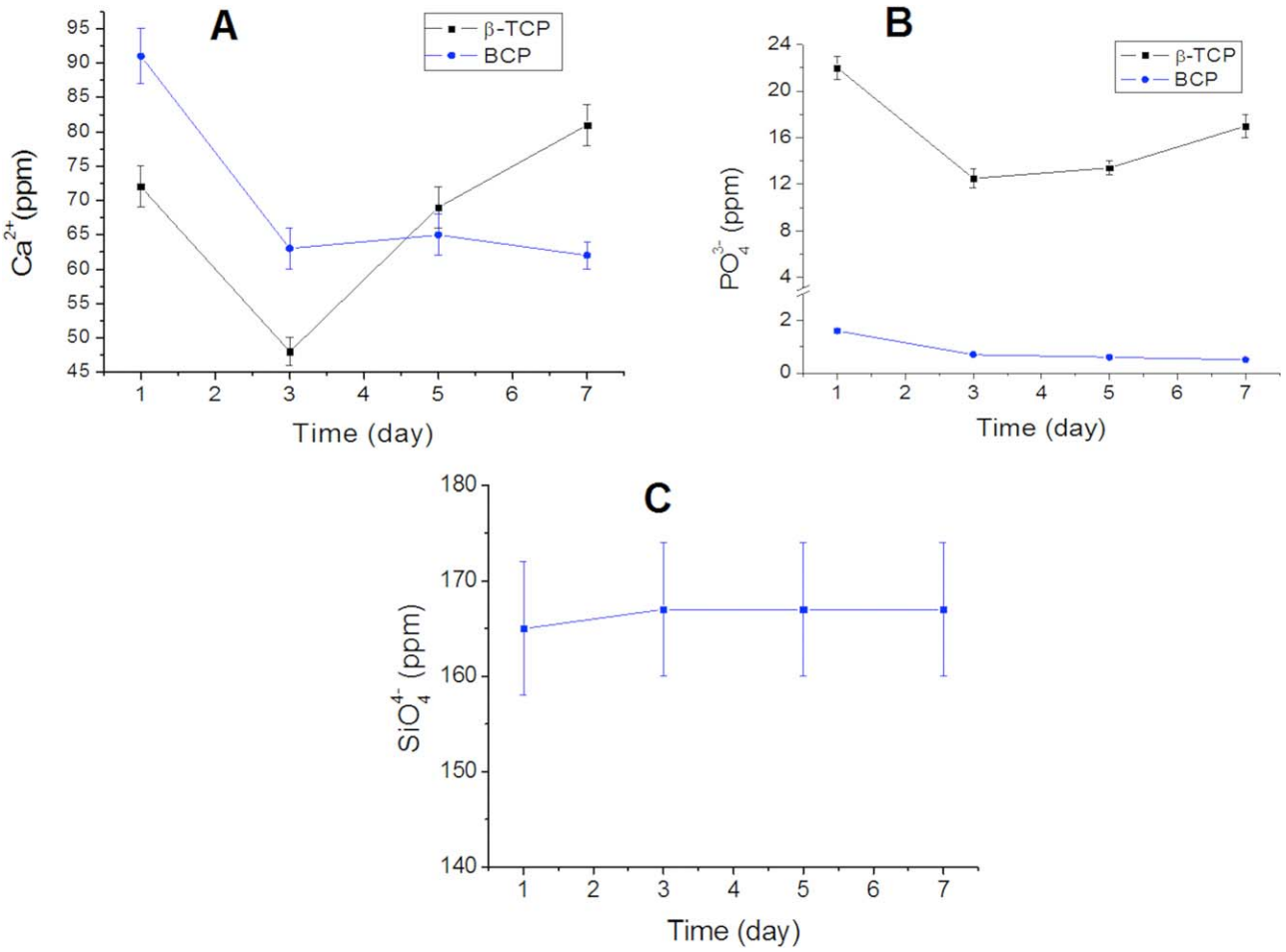

FIGURE 5. C_a^{2+}, SiO_4^{4-} and PO_4^{3-} release from β-TCP and BCP ceramics to DMEM during 7 days of incubation. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Figure 9 for healing periods of 8 and 12 weeks. The NB (%) values increased with time in both groups. A significantly higher percentage of newly formed bone was observed for BCP ceramic as compared to β-TCP ceramic. In BCP treated defects, newly formed bone was 61% at 8 weeks and 73% at 12 weeks. The percentage of residual graft material declined in the two cases at 12 weeks in comparison with the results obtained at 8 weeks. This percentage was much higher in the β-TCP ceramic than in the BCP ceramic at 12 weeks. The presence of fibrous tissue was higher in the case of control material at 8 and 12 weeks. Significant differences in percentage of new bone were found between the β-TCP and BCP at 8 and 12 weeks ($p < 0.05$).

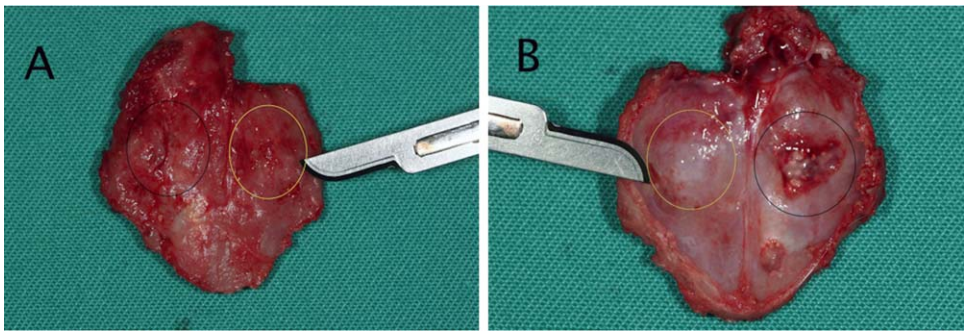

FIGURE 6. Macroscopically, it was evident the different behavior of tested ceramics at 12 weeks. External (A) and internal vision (B) of the rabbit calvaria. Yellow circle shows complete bone healing of the defect filled with BCP and black circle uncomplete healing defect filled with β-TCP. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

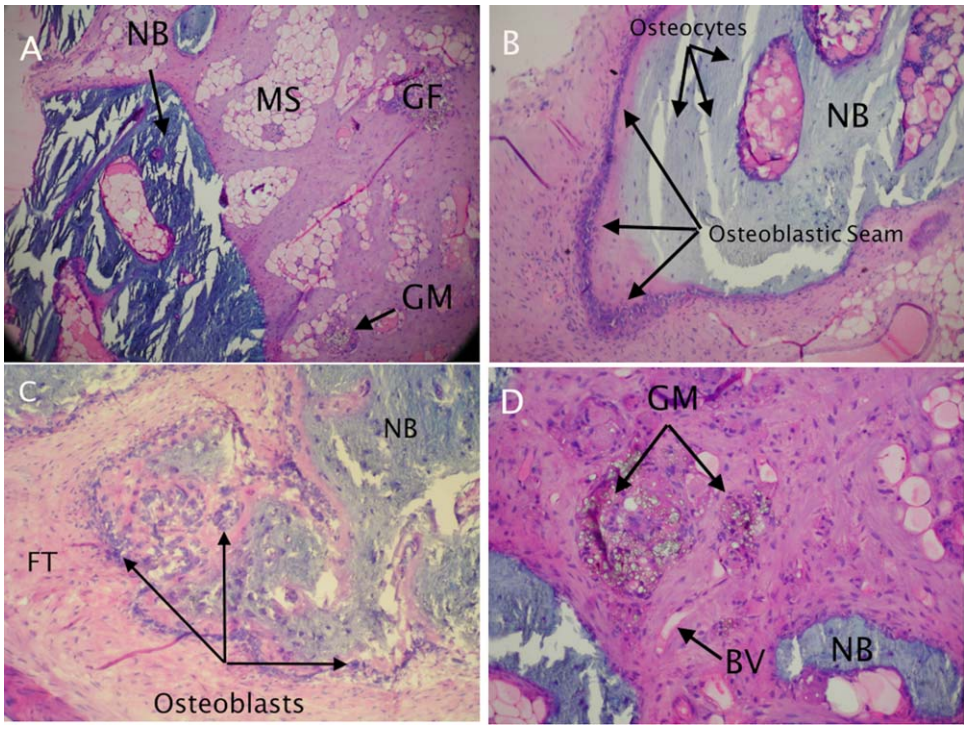

FIGURE 7. Histological sections of samples harvested after 8 weeks of healing. β-TCP (A and B) and BCP graft material (C and D). Graft material (GM), newly bone (NB), blood vessel (BV), marrow space (MS), and fibrous tissue (FT). Basic fuchsine and toluidine blue–stained sections. Magnification ×20. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

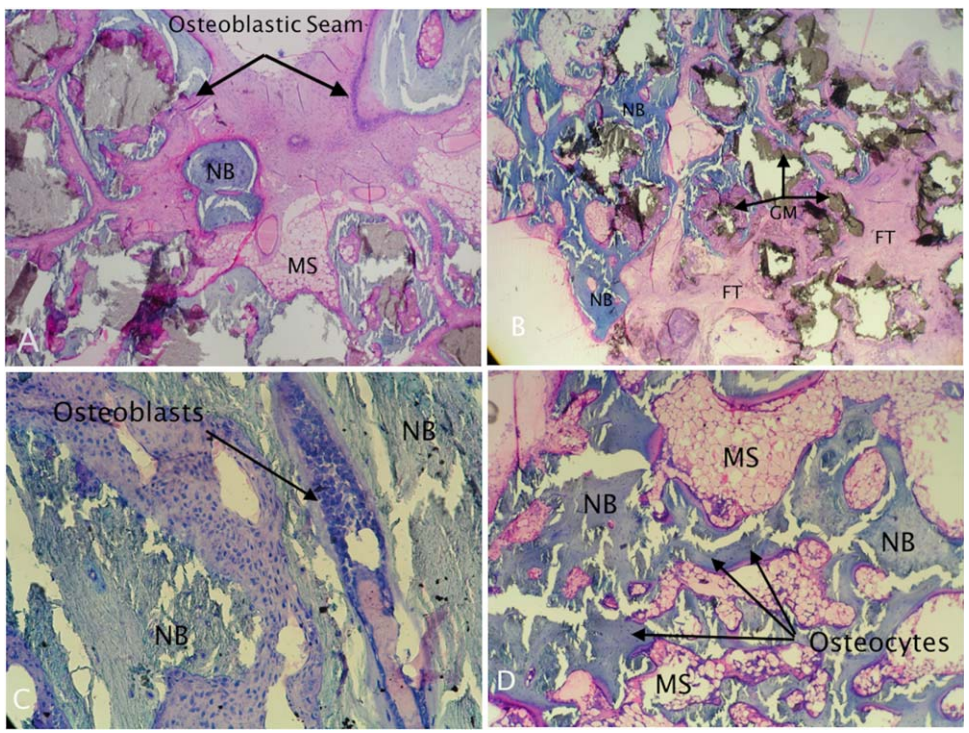

FIGURE 8. Histological sections of samples harvested after 12 weeks of healing. β-TCP (A, B) and BCP (C, and D). Abbreviations as in Figure 7. Basic fuchsin and toluidine blue–stained sections, magnification ×20. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

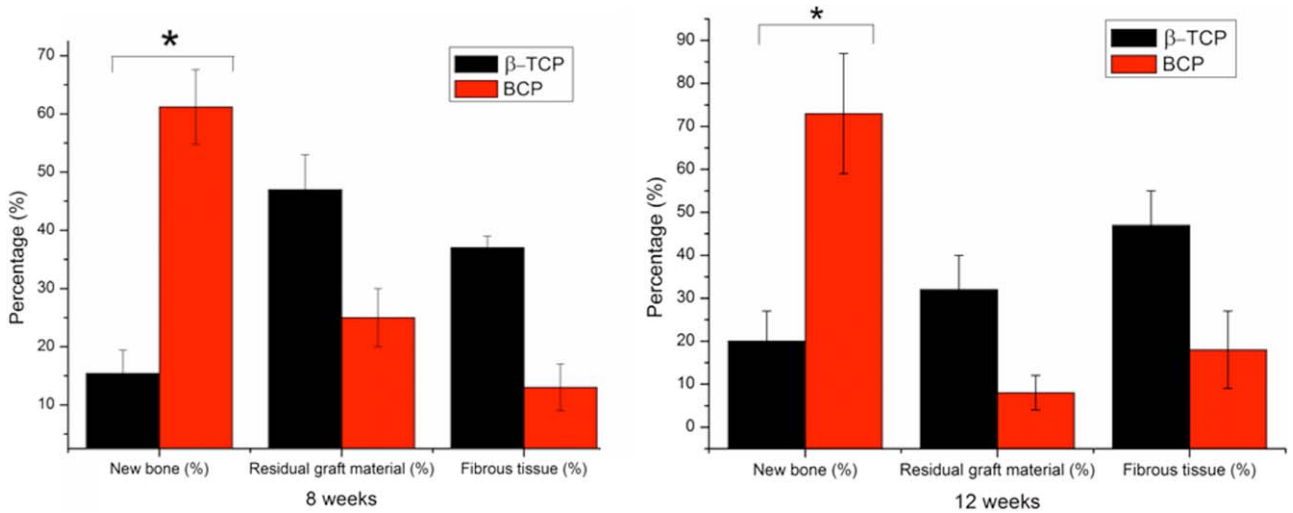

FIGURE 9. Percentage of newly formed bone, residual graft material and fibrous tissue in the defects treated with β-TCP and BCP at intermediate (left side) and late (right side) healing time. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

DISCUSSION

Ionic substitution plays an important role in the biological chemistry of bone apatite that has a similar crystallographic structure to hydroxyapatite ($Ca_5(PO_4)_3OH$). Various cationic (Ca, Sr, Mg, Na, K) and anionic substitutions (CO_3^{2-}) were included in the crystal of bone apatite.[13,31] These ionic substitutions resulted in microscopic crystals that are sufficiently insoluble for stability and sufficiently reactive to permit the remodelling process of resorption and reprecipitation *in vivo*.[32]

In this work, the incorporation of Si ions has successfully induced the synthesis of biphasic ceramic as indicated by X-ray diffraction analysis. The two mineral phases are β-TCP and calcium phosphate silicate mineral known as silicocarnotite ($Ca_5(PO_4)_2SiO_4$) that is a hydroxyapatite where a phosphate group is replaced by a silicate group. As a result of secondary reactions in the sintering process of temperatures above 700°C is obtained, induced by Si, a small amount of a stable calcium phosphate that can be identified as HA. HA is also obtained as a stable phosphate in the setting of β-TCP of ceramics doped with Si.[16,19] Furthermore, the presence of silicon ions induced significant morphological changes of the ceramics crystals as evident by SEM analysis (Fig. 2). The morphology was shifted from predominance of round-like grains in β-TCP to the predominance of plate-like grains in BCP ceramic. These morphological changes enhanced the cement SSA in the BCP ceramic, which could be related to the reduction in grain size and the presence of silicon-substituted hydroxyapatite that is known to have higher SSA.[33,34] Although the porosity was similar for both ceramics, the fraction of micro- and macropores were increased in the BCP ceramic.

The proliferation of osteoblast-like cells was significantly higher for BCP when compared to β-TCP. This improvement in cell proliferation and activity seems to be related to the release of SiO_4^{4-} ions from the BCP as both Ca^{2+} and PO_4^{3-} release form BCP was lower or equal to that of β-TCP (Fig. 5). Different studies have shown similar results for silicate ions on osteoblast cells by either using orthosilicic acid, degradation products of Si-containing materials (Bioglass, dicalcium silicate) or direct cell seeding on silicon-susbstituted ceramics.[16,18,23–27,35] This increase in osteoblast cells proliferation and activity could be mediated by the up-regulation of growth factors and genes that is necessary for osteoblast proliferation, extracellular matrix remodelling, and cell matrix attachment. Upregulation of proteins like transforming growth factor-β (TGF-β),[25] bone morphogenic protein 2 (BMP-2), and alkaline phosphatase (ALP) activity and genes encoded for osteocalcin, ALP, BMP-2 and Smad1 were reported.[24] Moreover, soluble silica has formerly been shown to play an essential role in the cross-linking of collagen and proteoglycans during bone growth.

In the present study, we tested the bone regeneration capacity of BCP ceramics, using as control β-TCP, by creating critical bone defects in the parietal bone of rabbits. Parietal bone has similar bone structure to that of jaw bone, as it is composed of two cortical plates and cancellous bone occupying the space in between. β-TCP ceramics are biocompatible, osteoconductive and their degradation rate is adequate for their replacement by the host osseous tissue.[18,35–39] Histological analysis revealed the absence of inflammatory reaction and, an adequate rate of resorption that permits the cells to fabricate the extracellular matrix and extend the area of newly formed bone (Figs. 7 and 8). This result agrees well with a previous observation of an almost complete resorption of β-TCP and its replacement by mature bone after 24 months of follow-up.[18]

The structural and morphological properties of BCP ceramics resulted in an enhanced new-bone formation and higher degradation than the β-TCP ceramic. The novel biphasic calcium phosphate ceramic was superior to the β-TCP in regard to the biological performance both *in vitro* and *in vivo*. BCP stimulated the production of significantly higher new bone formation and higher degradation rate

than β-TCP. This degradation, however, is compatible with the rate of bone deposition as the presence of fibrous tissue was limited. More mature bone was also observed in the defects treated with BCP. Silicon ions have an ionic radius of 0.41 Å, higher than that of phosphorus (0.34 Å), therefore Si—O bond length (0.161) is higher than P—O bond length (0,155), and thus the ionic radius of the phosphate group (PO_4^{3-}) is lower than that of the silicon group (SiO_4^{4-}). This may decrease the stability of calcium phosphates and thus increase their solubility, which may explain the higher degradability of the BCP ceramics.[40]

Among the amazing characteristics of autologous bone, the most striking is the efficiency to stimulate faster bone formation and its complete degradation. Thus, the novel BCP we synthesized, resembles at most these two properties of autologous bone, until now the best bone grafting biomaterial. The *in vivo* results indicated the almost complete regeneration of the critical bone defect by the BCP. Different authors have indicated higher bone neoformation in defects filled with β-TCP compared to those filled by xenografts,[18] meanwhile other studies, conducted in humans, showed regeneration rates similar to autologous grafts.[18,36] Histomorphometric results of the present study showed a 20% of the bone defect treated with β-TCP was filled by the newly formed bone at 12 weeks of healing. The highest amount of newly bone tissue was observed in bone defects treated with BCP, where 73% of the bone defect was filled by the newly formed after 12 weeks of healing. Macroscopically complete regeneration of the critical bone defects can be appreciated in those defects treated with BCP after 12 weeks of healing [Fig. 7(A,B)]. Statistically significant differences were found between β-TCP and BCP ceramics, and this result agrees well with that obtained by Kruse et al. with noncritical bone defects (6 mm diameter) produced in rabbit calvaria during a period of 4 weeks.[39]

CONCLUSIONS

A novel biphasic calcium phosphate was successfully developed to simulate the autologous bone behavior as graft material. A highly porous ceramic with enhanced specific surface area increased significantly the osteoblast cells proliferation and histological analysis showed the absence of inflammatory or immunological response to the novel biomaterial. The *in vivo* performance of the BCP resembles at most the autologous bone behavior, complete degradation and efficiency to stimulate new bone formation to regenerate critical bone defects.

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