Advances in Science and Technology Vol. 76 (2010) pp 66-71

doi:10.4028/www.scientific.net/AST.76.66

Osseointegration and biocompatibility study of macroporous biphasic calcium phosphate (BCP) ceramics obtained by consolidation using albumin

Christiane Ribeiro[1,a], Tamiye S. Goia[2,b], Kalan B. Violin[2,c], José C. Bressiani [2,d], Ana H. A. Bressiani [2,e]

[1] Universidade Federal do ABC- Centro de Engenharia, Modelagem e Ciências Sociais Aplicadas, Santo André,SP, 09210-170, Brazil
[2] Instituto de Pesquisas Energeticas e Nucleares, São Paulo, SP, 05508-000, Brazil
[a] christiane.ribeiro@ufabc.edu.br, [b] tsgoia@ipen.br, [c] kalanviolin@usp.br, [d] jbressia@ipen.br, [e] abressia@ipen.br

Key-words: macroporous ceramic, biphasic calcium phosphate, direct consolidation, albumin

Abstract: The potential of porous materials for applications in the medical, engineering and pharmaceutical areas has been widely reported. Several processing techniques have contributed to the progress in research involving porous biomaterials. To this purpose, a globular protein based (i.e. ovalbumin) consolidation approach has been proposed. In the present study, a porous hydroxyapatite: β-tricalcium phosphate - biphasic ceramics (BCP), was processed by direct consolidation using the protein-action technique. The processed porous ceramic exhibited appropriate pore configuration in terms of size, morphology and distribution. BCP cylindrical samples were implanted in male rabbits tibia to the evaluation of the initial biocompatibility and osseointegration for a 30 days period. The morphological analyses, optical microscopy and scanning electron microscopy evaluated the osseointegration. A rough surface pattern displayed by the ceramics seemed to have improved cell adhesion and proliferation processes. Furthermore, the open porosity of samples was an essential requirement for a suitable bone-implant osseointegration. In conclusion, this study revealed that the porous matrices obtained, promoted suitable development for bone tissue growth and also properties for osseoconduction and osseointegration.

Introduction

In the last years, the porosity in ceramic materials for implants obtaining has motivated the development of various technologies. Recent advances in colloidal processing method for near net shape ceramic have been incorporated into the biomedical field, to obtain more reliable porous grafts and scaffolds. Porous microstructure based biphasic calcium phosphate (BCP) ceramic are enable of promote variations in surface texture or microtopography and subsequent affect the cellular response to an implant [1, 2].

Studies aimed at a contribution to the development of advanced materials for use in reconstructive surgeries that require a high osteoconductive activity and potential application in the clinical area due to a relatively low cost of processing are always needed.

In terms of composition, Ca-P biomaterials are crystalline ceramics characterized by a high biocompatibility, an ability of direct bone bonding and osseoconduction, and a variable resorbability. Therefore, calcium phosphates materials have been considered as a priority within strategic focuses in the medical field. Such materials have been used in bioengineering, particularly in tissue engineering to obtain systems that induce the growth of bone cells (osteoinductive), and a great emphasis has been given to the use of biphasic Ca-P compositions [3].

Different studies have investigated the influences of pore size and geometry in induced bone formation in ceramics. They reported that microporous Ca-P structures have an increase surface area, which influences the biological behavior and the Ca-P dissolution/precipitation characteristics.

Macroporous dimensions promote highest amount of bone formation in implants and also an optimal vascular ingrowth. [4-6]. It has been reported that the phenotypic expression of bone cells cultured on bioactive ceramics was influenced by microstructure, surface roughness and general character of the material rather than their surface reactivity [7]. However, the better pore distribution and exact surface characteristics necessary for optimal osseointegration are still not fully understood.

The current work addresses the *in vivo* evaluation of bone-like structures fabricated with calcium phosphate biphasic (BCP) ceramic, which is the result of a recent development in processing of macroporous materials. These porous ceramics meet many of the requirements to enable their use as aids in reconstitution and substitution of damaged bone tissue and various other biomedical fields such as scaffolds for biomolecules cultivation, carriers for controlled delivery of drugs and matrices for tissue engineering.

Experimental Procedure

Macroporous samples of biphasic ceramic, based hydroxyapatite - HAp (Strem Chemicals) and β-tricalcium phosphate, β-TCP (Fluka) were produced by a novel route that comprises foaming and setting through protein action. In this method, a globular protein, albumin of eggwhite (ovalbumin) was used as a promising alternative, due its ability for pore formation and gelation in water [8, 9]. The procedure involves dispersion of an aqueous suspension of the HAp and β- TCP powder using as anionic dispersing agent (Dispex A40 -Ciba, an ammonium polyacrylate). Prior to this, the mixture was foamed by stirring with a double-blade mixer, aided by the addition of a nonionic surfactant (Genaminox, dimethyl alkyl amine oxide - Ciba) that reduces the surface tension of liquid-gas interfaces and stabilizes the foam. The albumin was incorporated into the suspensions to promote gelation by heat action. The bodies were cast into moulds and kept in oven at 60^{o}C/12 h and subsequently annealed at 700 ^{o}C/2h using a heating rate of 3 ^{o}C/min. in order to degrade of the organic material. The samples were sintered in oxidative atmosphere using heating rate of 5^{o}C/min up to 1250^{o}C for 30 minutes.

Scanning electron microscopy (Philips-XL 30) of gold-coated specimens was carried out for observation of the morphology of the porous structure. Pore size distribution was determined by mercury intrusion porosimetry (Micromeritics - Autopore III).

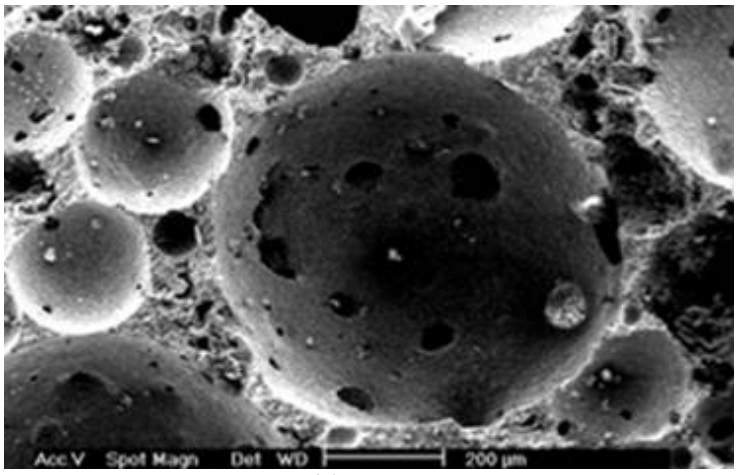
For the animal experiments, cylindrical specimens of biphasic ceramic foams with dimensions of 4 mm in diameter were cut from sintered bodies with a diamond-coated core-drill. Samples with porosity in the range of 73% vol were tested. For care and use of laboratory animals, the guidelines and regulations of Center of Biomedical Sciences, CB-IPEN, have been observed. Six adult albino New Zealand male rabbits weighing between 2.5 and 3.0 kg were anaesthetized by a intravenous femoral injection of 1mg/kg Rompun ^{TM} (Xylazyn Chloride) and 20mg/kg Hypnol ^{TM} (3% sodium pentobarbital). The animals were divided into three groups according to periods of sacrifice (7, 15 and 30 days). The area of implantation was shaved and prepared with iodine wash (Betadine^{TM}). Through an anteromedial approach into the third proximal of the tibia, three cortical bone defects were drilled with diameter of 4 mm. Two defects were filled with the HAp:β-TCP foam (Fig. 1) and the other acted as control. The periosteum and the skin were carefully closed with simple uninterrupted sutures. The periosteum was preserved in order to achieve better post-operative healing with bone remodelling. Antibiotic therapy (0.5 ml of benzilpenicylin of 1.200.00 U.i.n.) was carried out during the immediate postoperative period (48 h). Rabbits recovering from the surgical procedure were kept warm with a heating pad and observed until ambulatory. The implants were left for a maximum period of 4 weeks and at least 1 week, following a standard procedure [10]. The animals were sacrificed by an anesthetic overdose.

The area of implantation was sectioned and the tibial segments were immediately fixed with formaldehyde, one sample of with group was decalcified and the other was prepared in methyl methacrylate resin. The sections were stained with Hematoxilin-Eosin method for observation method for observation under optical microscopy.

Results and Discussion

Fig. 1 shows a representative specimen of the ceramic foams tested in this work. The structure appears to be typically composed of large spherical pores that result from the bubbles in the foam, thoroughly interconnected through open channels, all enclosed by a compact framework of polycrystalline hydroxyapatite : β-tricalcium phosphate ceramic.

In this technique, architecture of porous ceramic can be controlled and a completely interconnected structure with any desirable pore size and pore geometry is feasible. Obviously, interconnection of pores is essential for tissue growth throughout the porous matrix.



(a)

(b)

Figure 1. SEM micrograph of porous BCP ceramic based albumin and (b) BCP sample as implanted.

The foams were solidified in maturation stage, due to maintenance of cells with a spherical shape and absence of polyhedral geometry in the foam. This characteristic indicates that the rheology and dispersion condition of the suspension were well adjusted to have suitable stable foam, which is able to persist during solidification stage. SEM micrographs of the foams show that the structure consists of a permeable porous network, being observed spherical and interconnected pores, Fig. 1.

The porosity result determined using a mercury porosimeter is shown in Fig. 2 and porosity is expressed as volume of intruded mercury as a function of pore diameter. The samples presented a varied distribution of pores with spherical geometry and interconnected characteristic as regarded usually for implants.

The specimens revealed a narrow interconnection distribution in the range 1.0-5.0 µm and a wide distribution of interconnection in the range 30-200 µm. The small pore fraction seen in the specimens and observed in the micrographs of Fig.1 contribute directly towards inter-pore connectivity. Large pore size was not measure by mercury porosimetry because this technique shows the diameter of the interconnections. Interconnected pores are an important feature and this has been shown, in various studies, to contribute to and maintain bone tissue growth, by transport of nutrients and drainage of interstitial fluids [11- 14]. Pores with dimensions close to 5 µm can further facilitate ionic migration that happens initially between the surface of the implant and blood plasma, influencing favorably the cell absorption dynamics. These pores are also important in terms of

capillarity and permeability of body fluids, which could contribute to dissolution of this ceramic [11-14].

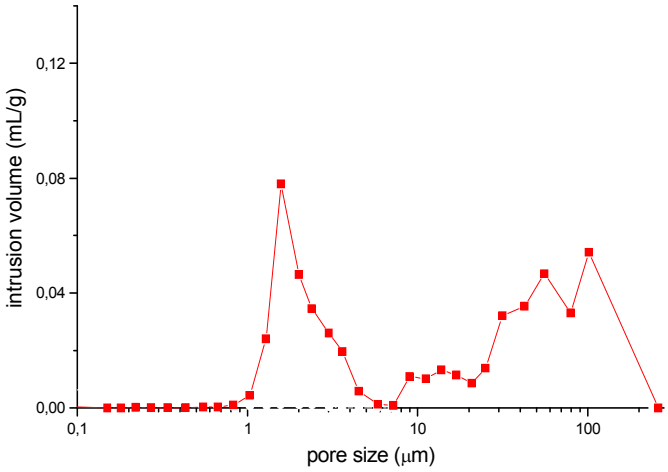
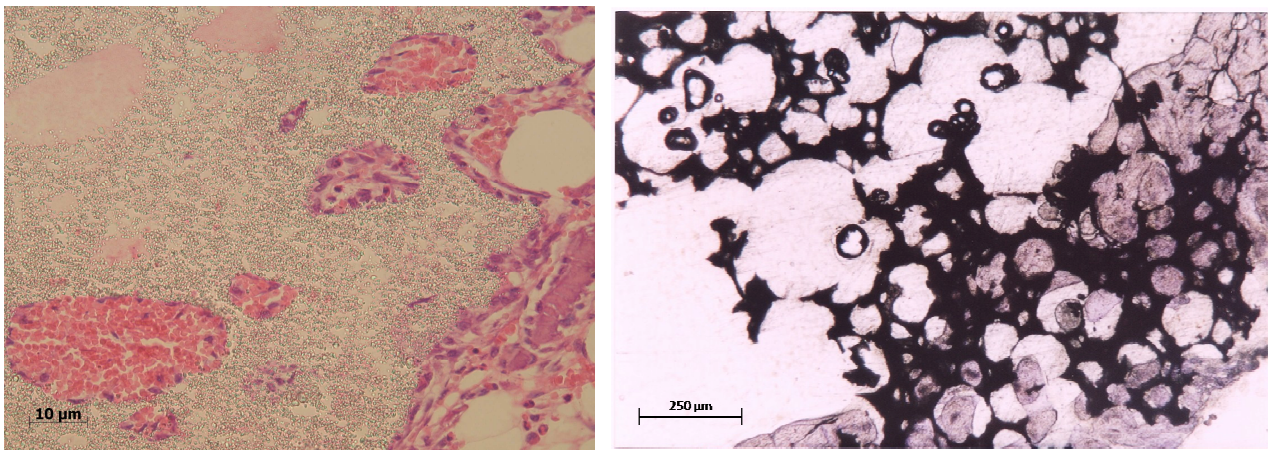


Figure 2- Pore size distribution curve of the porous BCP sample.

The macropores in these specimens provide paths for bone tissue growth, as the latter easily penetrate the pores and establish the osteoconduction process. The filling of these pores with new bone tissue gives strong interlaced bone-implant and confers increased strength to the implant.

A suitable porous network is required to promote extensive vascularization, for bone ingrowth, rapid bone regeneration and good implant integration. Bone ingrowth rates depend greatly upon the pore morphology, the degree of pore connectivity and pore volume. Pores larger than 100 μm are seen as necessary to allow blood and nutrient supply access for bone mineralization within the graft [13].

The micrograph presented in the Fig. 3 (a) shows pores completely filled with vascular tissue and amorphous substance, that will promote the cells differentiations and bone formation. A panoramic view of Fig. 3 (b), the biphasic ceramic implant (dark region) is being invaded by newly formed bone (violet region). The figure shows how the bone tissue in the large spherical pores of the samples advances through the open channels.



(a) (b)

Figure 3- Histological analysis with Hematoxilin-Eosin staining (a) osseointegration process in decalcified slide after one week and (b) panoramic viewof the bone ingrowth, calcified slide after 4 weeks of implantation.

Observations of the implant slides sections reveal an extensive ingrowth of new formed bone tissue into the pores of the BCP implants, Fig. 3 (b). New bone formation can be detected around and within the implant, thoroughly connected by bridges across the implant open pores.

The micrograph in the Fig. 3(b) demonstrates healing from the stage of unfilled pores at the outermost zones of the implant towards pores filled with organized tissue in the neighbouring the original bone, within the 4 weeks of implantation. The Fig. 3 (b) also depicts the tendency of osteon-structured bone to form mainly in areas neighbouring older bone.

These results illustrate bone-implant integration, new bone filling in the BCP macrostructure progressively, from areas of neighbouring old bone towards the inner part of the implant. In general, smaller pores are filled with bone more easily than larger pores, since the latter require correspondingly more cellular activity to be filled. In spite of this, larger pores have been reported to provide higher integration strength.

In all implants, bone tissue deposition occurred mainly in the form of layers with trabecular architecture and a significant number of osteocyte lacunae were noticed. Newly formed bone tissue at various stages of maturation was also detected. Fig. 3 (b) gives an example of mature bone with regular osteon structure surrounded by areas where the new bony tissue was present in later stages of maturation.

The macroporous biphasic ceramic presented in this work was filled almost entirely with trabecular bone within 4 weeks of implantation, confirming the high osseonconductive behaviour of HAp:β-TCP and the ability of the porous network to promote tissue ingrowth. Considering these results and the set of physical properties that characterises these materials, the macroporous ceramic demonstrate great potential as a structure for bone grafting. Furthermore, given that the process is applicable to a variety of compounds, the biphasic compositions may be chosen as desired, to achieve various degrees of resorbability and osseoconductive properties.

Conclusions

In vivo evaluation of macroporous biphasic ceramic (HAp:β-TCP) manufactured by the direct consolidation technique using albumin, carried out in the tibia of rabbits can be conclude that there was no evidence of undesired immune or inflammatory responses to the materials used in this study. The BCP samples allowed extensive osseointegration within 4 weeks implantation, indicating their potential for use as a scaffold for *in vivo* bone growth. The use of different raw materials with a similar porous structure to the one shown here can provide a variety of chemical and bioactive properties in order to suit a wide variety of applications, such as tissue engineering scaffolds and drug-delivery systems.

Acknowledgements

The authors are grateful to FAPESP and CNPq for financial support and to the Bioscience Center of the IPEN, particularly Dra. Olga Z. Higa by permission of the use optical microscope, allowing images acquired.

References

[1] B.D.Ratner, A.S. Hoffman, F.J. Schoen and J.E. Lemons, in: *Biomaterials Science, An introduction to Materials in medicine,*edited by Academic Press, San Diego (1996).

[2] Y. Xie, D.Chopin, C.Morin, Z. Zhu, J. Tang and J. Lu: Biomaterials Vol. 27 (2006), p. 2761–2767.

[3] D.D. Deligianni, N.D. Katsala, P.G. Koutsoukos and Y.F.Missirlis: Biomaterials Vol. 22 (2001), p.87-96.

[4] D.W. Murray, T. Rae and N. Rushton: J Bone Joint Surg. Vol.71-B (1989), p.632-637.

[5] C.B. Bozal. *Actualiz. Osteologia*. vol.2 (2006), p. 19-21.

[6] D. R. Villarreal, A. Sogal and J.L. Ong: J. Oral Implant Vol. 24 (1988) p. 67-73.

[7] D.M. Brunette: Int. J. Oral Maxillofac. Implants Vol.3 (1988), p. 231-246.

[8] C. Ribeiro, J.C. Bressiani and A.H.A. Bressiani: Key Eng. Mater. Vol.361-363, (2008), p.971-974.

[9] C. Ribeiro, J. C. Bressiani and A. H. A. Bressiani: Mat. Res. V. 10, (2007), p. 309-312.

[10] B. König Jr., T.J. Beck, H.F. Kappert, C.C. Kappert and T.S. Masuko: Ann. Anat. Vol. 180, (1998), p. 471-475.

[11] P. Ducheyne and Q. Qiu: Biomaterials Vol. 20 (1999), p.2287-2303.

[12] P. Sepulveda, A.H. Bressiani, J.C. Bressiani, L. Meseguer and B. König Jr.: Mat. Res. Vol.25. (2002), p. 253-256.

[13] A.Ravaglioli and A. Krajewski, in: Bioceramics: materials, properties, applications. Chapman & Hall, (1992).

[14] K.T. Bowers, J.C. Keller, B.A. Randolphi, D.G. Wick and C.M. Michaels: Int. J. Oral Maxillofac. Implants Vol.7 (1992),p. 302-310.