Key Engineering Materials Vols. 396-398 (2009) pp 27-30
online at http://www.scientific.net

Online available since 2008/Oct/21

In vitro characterization of porous ceramic based calcium phosphate processing with albumin

C. Ribeiro[1, a], W.I. Rojas-Cabrera[2,b], M. Marques[2], J.C. Bressiani[1], A.H.A Bressiani[1,c]

[1] Instituto de Pesquisas Energéticas e Nucleares - IPEN, Av. Prof. Lineu Prestes, 2242, Cid. Universitária, 05508-000, São Paulo, SP-Brasil.

[2] Faculdade de Odontologia - Universidade de São Paulo, FOUSP, Av. Prof. Lineu Prestes, 2227, Cid. Universitária, São Paulo, SP-Brasil.

[a] cribeiro@ipen.br, [b] israelrc@usp.br, [c] abressia@ipen.br

Keywords: porous ceramic, calcium phosphate, *in vitro* characterization

Abstract. In recent years, the processing of porous ceramic materials for implant applications has motivated the development and optimization of new technologies. 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 consolidation using the protein-action technique. The processed ceramic materials exhibited appropriate pore configuration in terms of size, morphology and distribution. The *in vitro* reactivity and dissolution behavior of the ceramics was evaluated in SBF and biocompatibility in an osteoblasts culture, respectively. Overall, the materials tested showed biocompatibility and suitable properties for osteoconduction. A rough surface pattern displayed by the ceramics seemed to have improved both; cell adhesion and proliferation processes. In conclusion, this study revealed that the porous matrices obtained, promoted suitable development of cell metabolism without cellular death.

Introduction

Biphasic calcium phosphate ceramics (BCPs), particularly composed of hydroxyapatite (HAp) and β-tricalcium phosphate (β-TCP) are considered as suitable materials to be used for bone grafting procedures. It has been reported that BCPs exhibit chemical and physical characteristics that are designed to meet specific application requirements [1].

The processing of porous matrices based on these calcium phosphate ceramics may induce a suitable attachment and proliferation to osteoblasts, which is required by material design guidelines. Porous microstructure based BCP ceramics are also able to create surface texture and microtopography, which subsequently affects the cellular response to an implant [2]. It has been reported that the phenotypic expression of bone cells cultured on bioactive ceramics is influenced by microstructure, surface roughness and general character of the material, rather than their surface reactivity [3, 4]. However, the optimum pore distribution and surface characteristics necessary for osteointegration are still unclear.

The objectives of this study are to evaluate: the reactivity of porous biphasic ceramics in SBF medium, and to analyze the *in vitro* behavior of osteoblasts with porous BCPs.

Materials and Methods

BCP ceramics (HAp:β-TCP) with porosity of about 70% were obtained using a consolidation methodology proposed elsewhere [5]. The *in vitro* reactivity and dissolution tests were analyzed by soaking the samples in a SBF solution, which was maintained at 37 °C under agitation (40 rpm), for a maximum period of 21 days; the solution being replenished every 3 days. Sample surfaces were examined using a scanning electron microscope (SEM-EDS, Philips-XL 30), before and after

immersion in SBF. The *in vitro* biocompatibility was investigated by osteoblasts culture (OSTEO-1) in a Dulbecco's modified Eagle's Medium (DMEM), (FBS, Gibco, USA) supplemented with 10% fetal bovine serum and 1% antibiotic-antimycotic solution. The cells were incubated at 37 °C in a humidified 5% CO_2 and 95% air atmosphere. Osteoblasts culture were tested in triplicate, on BCP samples sterilized by gamma radiation (25kGy), following analysis for 0.5; 1; 2 and 3 days on each sample. The surfaces and biocompatibility were examined by SEM. The proliferation behavior and morphological analysis was performed during this study.

Results and Discussion

During the study in SBF, the deposition of a bioactive layer with a globular shape typical of the $Ca_{10}(PO_4)_6CO_3$ was observed, Fig.1. Porosity was found in the BCP microstructure, as shown in Fig.1-d and 1-e, which generates a larger contact area, suitable for liquid permeation, causing an accelerated SBF medium saturation.

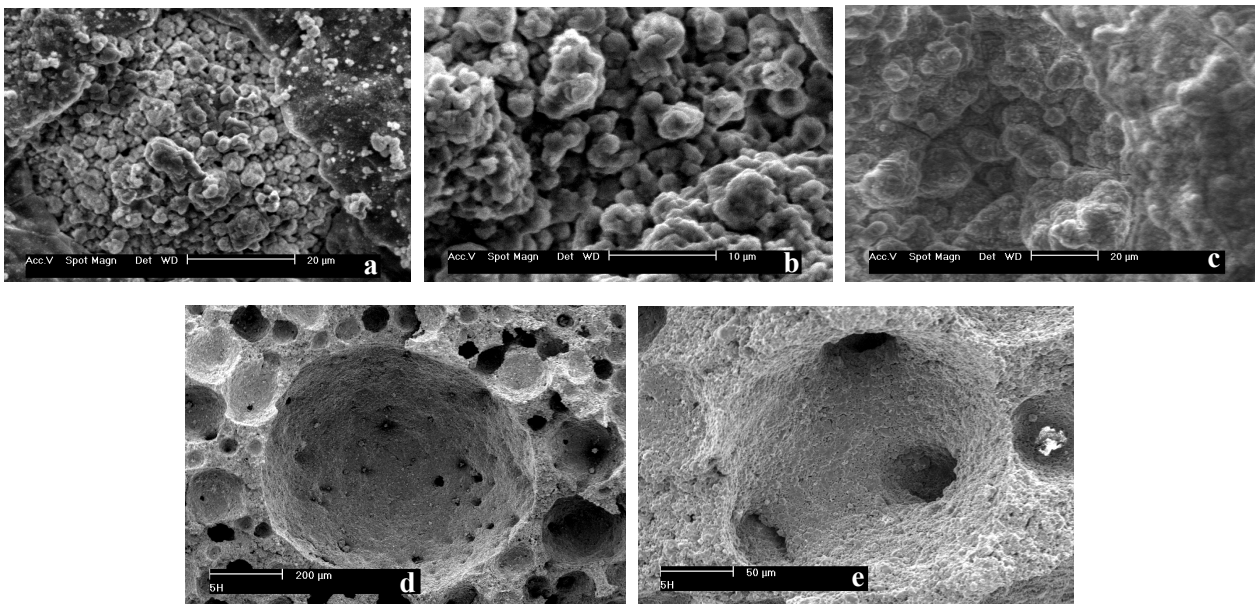

Figure 1 - SEM micrographs showing the surface of the samples before (d and e) and after of the soaking in SBF at different intervals: (a) 15 days; (b and c) 21 days.

In vitro reactivity and dissolution behavior was noticed, with a pH variation of 19.3% ± 0.5, in the alkaline range and an increased mass of 3.7% ± 0.2 at every renewal (3 days) of SBF medium.

Substantial anchorage of osteoblasts was observed in the porous ceramics surface, Fig.2. The anchorage was associated to the presence of an adhesive glycoprotein (osteopontin) that assists in the adhesion, migration and cell proliferation processes. Interestingly, osteopontin has higher affinity for the HAp [6].

Processing induced porosity ultimately dictates the morphological variations of osteoblasts, Fig.2-a, clearly due to the evolution of different sample topographies. Osteoblasts presented elongated phenotype with stellar shape and long cytoplasmatic prolongations. The presence of pseudopodes or phyllopodia, structures that are representatives of good adhesion in osteoblasts were also observed (Fig.2-a, b). It is a well-known fact that in porous materials and irregular surfaces, the presence of cytoplasmatic prolongations is more likely than phyllopodia [7]. These cytoplasmatic prolongations are likely to effect the formation of lacunae and osteocitary channels, which often form an interconnected network, known as lacuno–canicular system, aiding the diffusion of nutrients and interstitial liquids [8].

During the first few hours, the cells showed low activity, a characteristic associated with the flattened and fine aspect of the cell membrane, but with suitable attachment. A trend of anchorage

on shall of the pores was observed, which also maintained contact through multiple prolongations with neighboring cells. However, a confluence period was not noticed during this study.

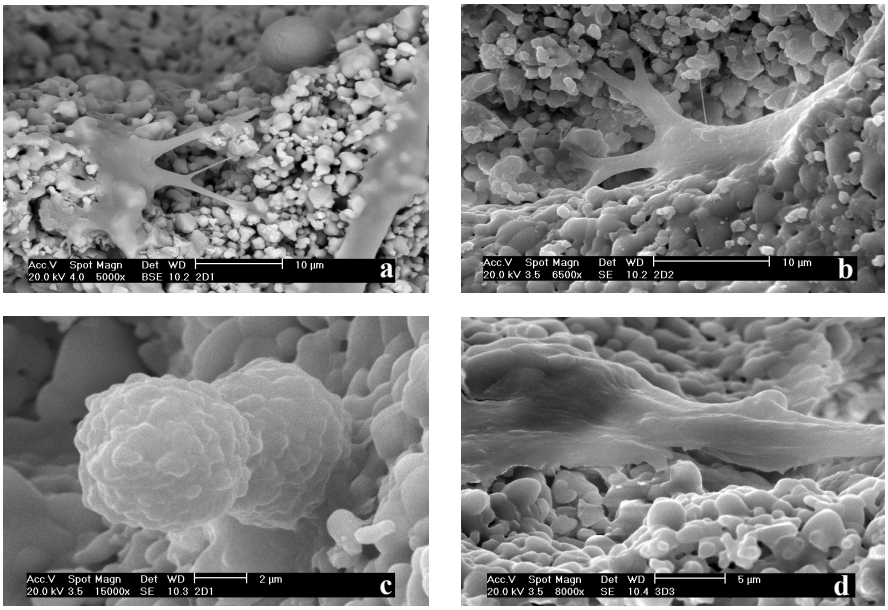

Figure 2- SEM micrographs showing the morphology and behavior of cells on ceramic in different incubation periods: (a, b and c) 2 days and (d) 3 days.

After 2 and 3 days, the cells exhibited a dense membrane (Fig. 2-d) and high activity, a characteristic that is reinforced by the rounded shape of the osteoblasts and vesicles (Fig. 2-c). This morphology is typical for cells that grow in monolayer, energetically favorable to the cell division process. During the cell growth phase, they showed an accelerated metabolism, because they are susceptible to pH, temperature, pO_2 and pCO_2 variations [8].

Fig.3 shows the cell viability variation with time, in a maximum at 3 days (72 hours), revealing osteoblasts behavior. The number of cells on BCP samples increased with time. Suppression period, which reveals adverse reaction or cytotoxicity was not observed among the cultures, Fig 3.

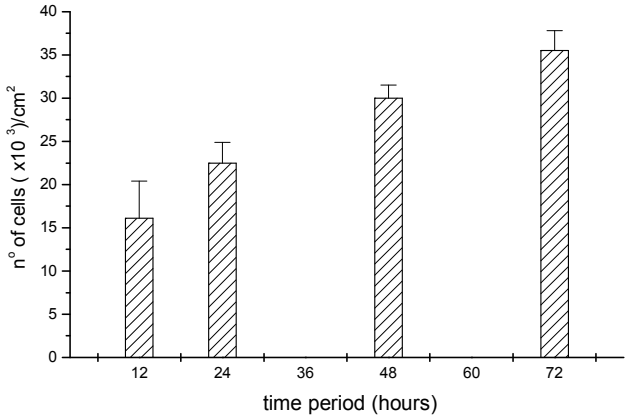

Figure 3- Cell viability graph, showing the biocompatible nature of the samples.

During the first 12 hours, the cell metabolism most likely was associated with the anchorage process, as suggested by good adhesion behavior. The sample-surface roughness may have brought about an increase in the contact surface area for each cell, and consequently improved adhesion with BCP. Cell anchorage requires specific adhesive proteins, while proliferation and differentiation needs growth factors and cytokines that assit cellular intercommunication [6]. After adhesion cells initiate the proliferation/differentiation process.

During the latter period of the analysis, the metabolic functions were involved to cellular differentiation process. Therefore, an expressive increase of the number of cells was not observed in samples cultured for 2 and 3 days, Fig. 3. Additionally, evidence of phenotype characteristics was observed, similar to samples shown in Fig.2 (a), (b), likely due to cytoskeleton reorganization, position of organelles, diffusion of intracellular components, cell form modeling and cellular differentiation [8].

Samples cultured for 2 and 3 days showed an increase in number of cell-to-cell contacts, establishing further interconnections and evidence of an underlying mitosis process, as shown in Fig. 2-c. In this period the cell grows, only if it differentiates and is able to repair itself [7].

The size increase of some cells could actually be a morphologic alteration, prevalent in samples analyzed for 2 and 3 days. Studies have reported a strong mutual relationship between osteoblast and different implant surfaces (e.g. HAp), with an obvious dependence on the topography and nature of surface where they are cultured [3, 9]. The morphology of osteoblasts cultured on porous ceramics was reported to be significantly different than those cultured on smooth surfaces [4]. The discernible differences in morphology of osteoblasts can be attributed to the heterogeneous topography possessed by the ceramic. This irregular characteristic of the surface can often result in pressure variations, due to fluctuation in O_2 and CO_2 levels at the top surface, stimulating of different forms the cell metabolism.

Conclusions

The *in vitro* reactivity tests showed an accelerated dissolution of the BCP ceramics in the presence of SBF. The cell culture test results showed that osteoblasts exhibit good interaction with BCP porous ceramics, suggesting high preference of adhesion for irregular surfaces. In addition, the synergism of macrostructure with chemical composition of the ceramic seems to promote suitable attachment and cellular response, and this could lead to high expectations as bone substitutes or scaffolds for tissue engineering.

Acknowledgements

The authors are grateful to CNPq and FAPESP for financial support.

References

[1] B.D.Ratner, A.S. Hoffman, F.J. Schoen, 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, J. Lu. *Biomaterials*. vol. 27 (2006), p. 2761–2767.

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

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

[5] C. Ribeiro, J.C. Bressiani, A.H.A. Bressiani. *Key Eng. Mater*. V.361-363, (2008), p.971-974.

[6] D. R. Villarreal, A. Sogal, 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.B. Bozal. *Actualiz. Osteologia*.Vol.2 (2006), p. 19-21.

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

Bioceramics 21
doi:10.4028/www.scientific.net/KEM.396-398

In Vitro Characterization of Porous Ceramic Based Calcium Phosphate Processing with Albumin
doi:10.4028/www.scientific.net/KEM.396-398.27

References

[1] B.D.Ratner, A.S. Hoffman, F.J. Schoen, 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, J. Lu. Biomaterials. vol. 27 (2006), p. 2761–2767.
doi:10.1016/j.biomaterials.2005.12.011
PMid:16426679

[3] D.D.Deligianni, N.D.Katsala, P.G.Koutsoukos, Y.F.Missirlis.Biomaterials v.22 (2001), p.87-96.
doi:10.1016/S0142-9612(00)00174-5
PMid:11085388

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

[5] C. Ribeiro, J.C. Bressiani, A.H.A. Bressiani. Key Eng. Mater. V.361-363, (2008), p.971-974.
doi:10.4028/www.scientific.net/KEM.361-363.971

[6] D. R. Villarreal, A. Sogal, J.L. Ong. J. Oral Implant. Vol. 24 (1988) p. 67-73.
doi:10.1563/1548-1336(1998)024<0067:PAAORT>2.3.CO;2
PMid:9835832

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

[8] C.B. Bozal. Actualiz. Osteologia.Vol.2 (2006), p. 19-21.

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