Key Engineering Materials Vols. 330-332 (2007) pp. 935-937
online at http://www.scientific.net
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In Vitro Evaluation of Porous Biphasic Scaffolds

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Keywords: Porous scaffolds, hydroxyapatite, beta tricalcium phosphate, simulated body fluid.

Abstract. In the present study, two methods for the production of three-dimensional scaffolds made of bioactive ceramics are presented. Depending on the method, the final product can be composed by pure hydroxyapatite or biphasic: hydroxiapatite + tricalcium phosphate. Bioactivity tests showed that all scaffolds are bioactive. Preliminary studies with adipose stem cells indicated biocompatibility of both scaffolds.

Introduction

Porous bioceramics for bone regeneration and tissue engineering have been produced by several techniques such as the use of polymeric sponge, foaming processes and techniques using organic additives [1-3]. These materials must allow bone in growth, when used as bone filler. For tissue engineering, the scaffolds must be a three-dimensional substrate to support cells undergoing events of spreading, proliferation are differentiation. It is well known that the pores must be interconnected [1-3]. Pore sizes depend on the type of cells used and on the material. If the bioceramic is a bioresorbable one, like tricalcium phosphate (TCP), it is assumed that pores interconnections will undergo dissolution, allowing larger pores.

Materials and Methods

In the present study, porous hydroxyapatite scaffolds were produced by two different techniques. The three-dimensional bodies were characterized by Scanning electron microscopy (SEM) and X-ray diffraction (XRD). *In vitro* characterization was performed with simulated body fluid and in cell culture using induced adipose stem cells.

In the first method, stoichiometric hydroxyapatite was mixed with polyethylene wax spheres (Clariant, Ltda.) in a proportion of 75% vol. wax spheres. The mixture was uniaxially pressed at 40MPa to produce 10mm discs. The elimination of the organics was performed by heat treating at 550ºC for 2 hours with a heating rate of 0.5ºC/min. The consolidation of the bodies by sintering was achieved at 1100ºC during 1 hour, at a heating rate of 5ºC/min.

In the second method, polyurethane sponges were coated with calcium deficient hydroxyapatite from a solution rich in calcium and phosphate ions. The struts were dried in an oven at 60ºC. The sintering path was the same for all the specimens.

The samples were incubated in simulated body fluid, following the procedure proposed by Kokubo.

Preliminary tests with adipose stem cells (ASC) were performed in order to assess the capacity of adhering and spreading in the three-dimensional scaffolds. ASC were obtained from patients undergoing liposuction procedures and cultured in osteoblast differentiation medium (ODM). The ASC cells were seeded on the biphasic scaffolds in a number of 3.3×10^5 cells and cells were cultured during 7 days, with medium exchange every third day.

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Results and Discussion

Figure 1 shows SEM pictures of the samples produced with wax spheres. Several closed pores can be observed, in the addition to the interconnected porosity.

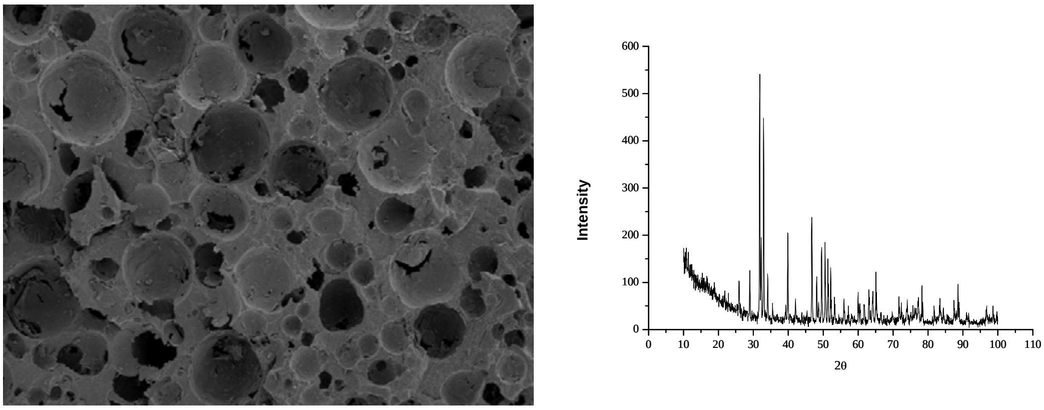

Figure 1 – Hydroxyapatite sample produced by wax spheres addition and corresponding XRD pattern.

Figure 2 shows the three-dimensional scaffolds produced by the polymeric sponge method. A completely interconnected structure can be observed.

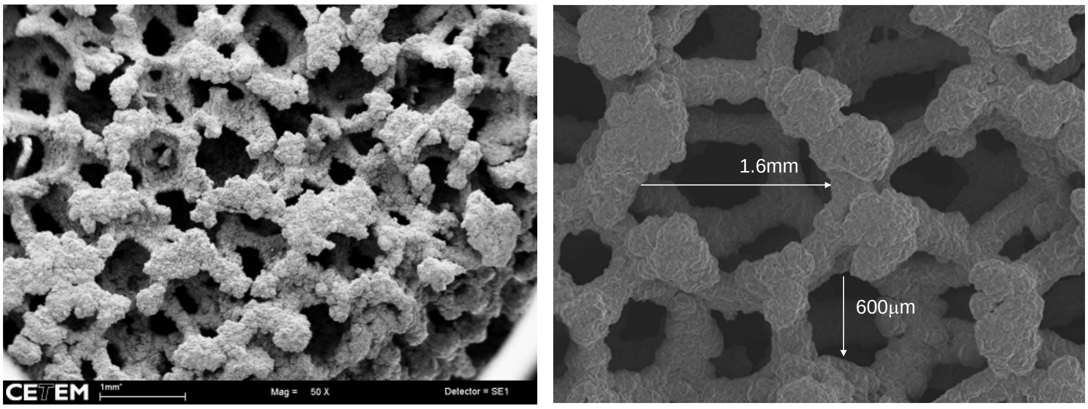

Figure 2 – SEM images showing the three-dimensional scaffolds produced by the polymeric sponge coating method.

XRD analysis of the samples produced by the polymeric sponge method showed that the scaffolds are composed of hydroxyapatite and β-tricalcium phosphate, β-TCP, as shown in Figure 3. After incubation in simulated body fluid, the samples produced by both methods exhibited precipitation of bone-like apatite, as shown in Figure 4.

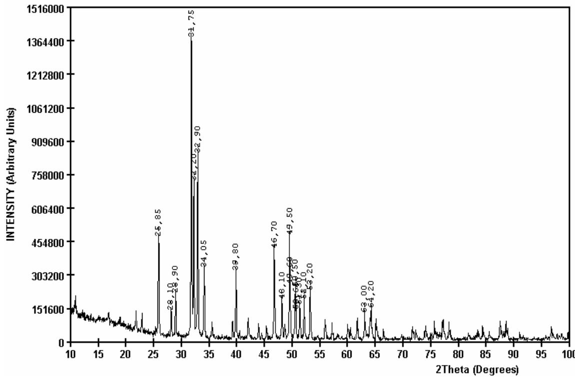

Figure 3 – XRD analysis of the polymeric method scaffolds, confirming the presence of biphasic ceramics HA + β-TCP.

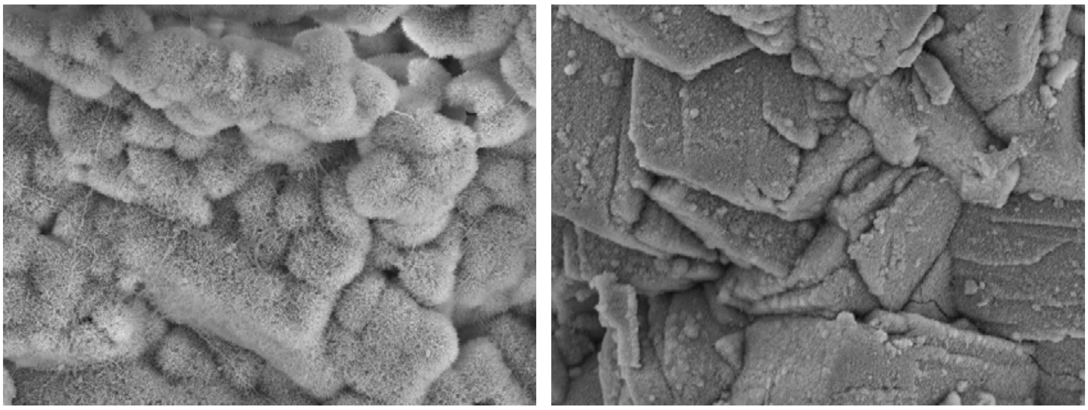

Figure 4 – SEM images showing bioactivity of scaffolds produced by wax spheres addition with 2000X magnification (a) and the polymeric sponge coating method (b), with 1000X magnification.

Figure 5 shows the preliminary results of cell culture using adipose stem cells, showing that the cells adhered and proliferated on both scaffolds. The hydroxyapatite struts, produced with wax spheres, and HA + β-TCP struts, produced by the polymeric sponge method, provided a suitable surface for cells spreading, indicating the biocompatibility of the scaffolds.

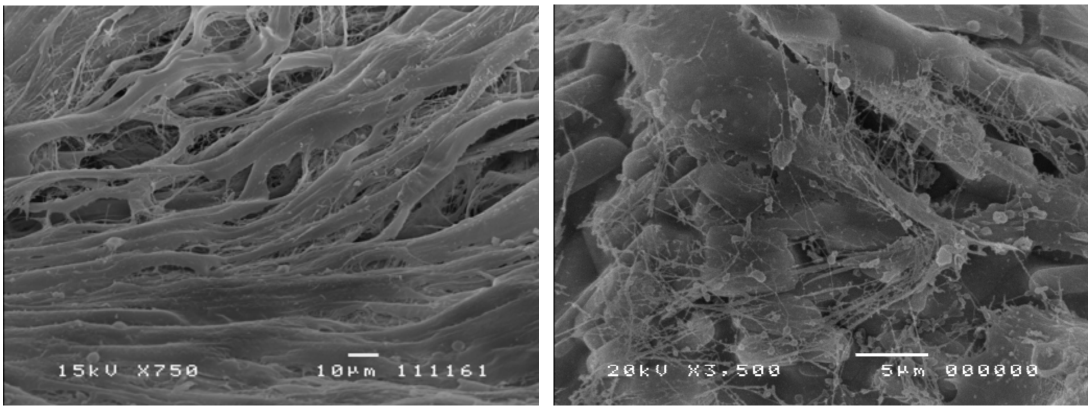

Figure 5 – Adipose stem cells cultured on hydroxyapatite scaffold (a) and biphasic HA + b-TCP scaffold, showing biocompatibility.

Conclusions

Both methods are able to produce bioactive and biocompatible tridimensional scaffolds. However, the polymeric sponge coating method produced totally interconnected struts. These struts present bioactivity and are biphasic. This result is promising, as the conjugation of phases with different solubility is a key factor for the production of scaffolds with dissolution rates compatible with the new bone formation one.

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