J Mater Sci (2010) 45:4990–4993
DOI 10.1007/s10853-010-4363-1

ICAM 2009

Scaffolds of poly (ε-caprolactone) with whiskers of hydroxyapatite

G. B. C. Cardoso · S. L. F. Ramos · A. C. D. Rodas · O. Z. Higa · C. A. C. Zavaglia · A. C. F. Arruda

Received: 18 November 2009 / Accepted: 27 February 2010 / Published online: 18 March 2010

Abstract Scaffolds of Poly (ε-caprolactone)/hydroxyapatite were produced and studied for tissue engineering applications. The materials were selected due to its biodegradability (PCL) and bioactivity (HA), and above all their biocompatibility toward the human tissue. The composites produced were characterized by SEM, XRD, and EDS. By analyzing these characterizations it was possible to obtain further information about the composition and morphology aspects of all portions of the composite scaffold.

Introduction

Along with the great advances of medicine, in terms of development of new and more specific drugs, and the discovery of chemical interactions and cell regeneration capabilities, came the need to develop novel materials for medical usage. They are meant to act in many ways, such as: drug delivery systems, mechanical support, cell regeneration platforms, replacement of specialized tissues, healing assistance, and even cosmetic correction. Given this point of view, it is easy to infer that to develop these fully functional materials, one must comprehend a vast field of knowledge or collaborate with several different specialties, since this involves areas of Engineering, Physics, Chemistry, Medicine, Biology, and Material Science.

More specifically, due to the high quantity of accidents, defects, and diseases that affect the human bone structure, intensive research has been dedicated to this matter. A great variety of materials and processes was developed or adapted so that substitute materials and engineered tissues of sufficiently high fidelity can be produced to help address the growing problem of bone tissue and organ failure.

Poly (ε-caprolactone) (PCL), is a biodegradable and bioreabsorbable polymer which has a good biocompatibility, and it is approved by the Food and Drug Administration (FDA) as a biomaterial. This polymer has been used in many researches, above all, in tissue engineering, because of its success in the last years, and its easy processability and formulation with respect to the traditional biocompatible metal or ceramic implants [1, 2].

Scaffolds are manmade three-dimensional structures utilized as supports for cell growth and proliferation. These structures are very particular and very difficult to be produced, once it must attend several properties, mainly mechanical and chemical, that are specific to the tissue to be treated. According to the tissue that these scaffolds must attend, they have a slight alteration on the main properties required. Specifically for bone engineering applications, the ideal scaffold must be biocompatible to the human fluids and tissue, osteoinductive, osteoconductive, and mechanically compatible with the native bone. These are the properties that will provide cellular anchorage sites, mechanical stability, and structural guidance to the new developed tissue [3].

G. B. C. Cardoso (✉) · S. L. F. Ramos · C. A. C. Zavaglia
Department of Materials Engineering, Faculty of Mechanical Engineering, State University of Campinas, Campinas 13083-860, Brazil
e-mail: guicardoso@fem.unicamp.br

A. C. D. Rodas · O. Z. Higa
Center of Biotechnology, IPEN, São Paulo 05508-000, Brazil

A. C. F. Arruda
Department of Petroleum Engineering, Faculty of Mechanical Engineering, State University of Campinas, Campinas 13083-860, Brazil

PCL scaffolds present a good bioreabsorption rate that is appropriate for bone tissue regeneration, and also provide suitable mechanical properties during a sufficient time. Unfortunately, PCL has an intrinsic hydrophobic chemical nature, and to decrease this property it was made a ceramic addition. This ceramic has a main role that is to provide a more bioactive substrate, were bone cells can easily recognize and develop above the material [4].

When a composite is thought of, and in this case utilizing inorganic fillers into a polymeric matrix, the main objective is to either improve its mechanical properties or to reduce cost for its production. As for the improvement of mechanical properties, the objective is to produce structures biomechanically compatible to the target tissue [5]. Also, these fillers must have some basic characteristics to be effective. First of all, their particle size must be smaller than the interchain distances inside the polymers, so that it will not generate points of stress concentration in the material. Also, there will be variation of effectiveness according to the interaction between the surface of the filler and the polymer, aspect ratio of the filler, and compatibility of filler and polymer, once the main purpose is to transfer loads through the polymer phase to the filler, which have a higher modulus. Generally, these fillers are chemically treated, so that they can increase their surface area and have a better anchoring with the polymer matrix.

In the case of biomedical composites, not only the classical rules of composites materials are applied, making the properties of these materials much more controllable, but also, they can be used to improve biological responses, carrying specific drugs, bioactive factors, among others. In fact, it is very important that the mechanical properties of biomedical materials are improved, especially for the fabrication of scaffolds and bone substitute materials. In this case, these biomedical composites should carry at least biocompatible properties, not stimulating immunological responses toward the material, and suitable mechanical properties, to be able to support the minimum loads of the specific tissue. Therefore, the main goal in this research was to develop polycaprolactone/hydroxyapatite scaffolds, using the solvent-casting particle-leaching of PCL, and the ceramic filler was produced by alkaline hydrolysis of alpha- tricalcium phosphate (α-TCP).

Experiment

Ceramics

In order to produce [alpha]- tricalcium phosphate (α-TCP), lab made reagents were utilized: Calcium Hydrogen Phosphate ($CaHPO_4$) and Calcium Carbonate ($CaCO_3$), produced by aqueous precipitation method [6]. Afterwards, a mixture of both reagents was heated in a rate of 5 °C per minute up to 1225 °C in a Lindberg/Bluem furnace, resulting in the syntheses of α-TCP by solid state reaction.

The aqueous hydrolysis of α-TCP occurs during 6 h under a temperature of 90 °C. The pH was controlled with the addition of ammonium hydroxide (NH_4OH, from Synth, Brazil) in the value of 11 [7].

Composite

The scaffold was made using poly (ε-caprolactone) (PCL, from Aldrich), with molecular weight of 80 kDa, and dissolved in chloroform ($CHCl_3$, Merck) by stirring for 6 h. In order to produce porous scaffolds, the salt-leaching method was utilized. The porogen agent used was the sodium chloride (NaCl, from Synth, Brazil) in a size of 177–350 μm [8].

The addition of the hydroxyapatite whiskers was made before the 6 h of stirring. The proportion filler/polymer was tested in others works and the one selected was 0.2 g HA for 1.5 g PCL [8].

After 48 h for the evaporation of the solvent, the samples were washed in deionizer water during 48 h for the porogen leaching. The samples were then dried in vacuum, and kept until the use.

Instrumental characterization

The morphology of the ceramic filler was observed by images made with scanning electron microscopy (SEM) and the calcium phosphate rate was obtained by the analysis of X-ray fluorescence (FRX). To confirm the ceramic pattern the X-ray diffraction (XRD) was utilized, were for the α-TCP, the pattern was 09–0348 and for the hydroxyapatite whiskers, 09–0432.

The resulting composite was also analyzed by scanning electron microscopy (SEM) in order to see the disposition of the filler within the polymeric matrix. Finally, X-ray diffraction (XRD) was used to observe peaks of crystalline PCL and hydroxyapatite in the final composite.

Results and discussion

The crystalline phase of the α-TCP was investigated by XRD and identified as α phase with traces of β phase peaks (Fig. 1a). As for the HA whiskers, a clear HA pattern was obtained, with also good crystallinity and high purity, as shown in Fig. 1b. The Ca/P ratio of hydroxyapatite whiskers was 1.62, consistent with non-stoichiometric, Ca-deficient HA.

Observing the SEM images, the hydroxyapatite whiskers appeared to be aggregates of a great number of fibers

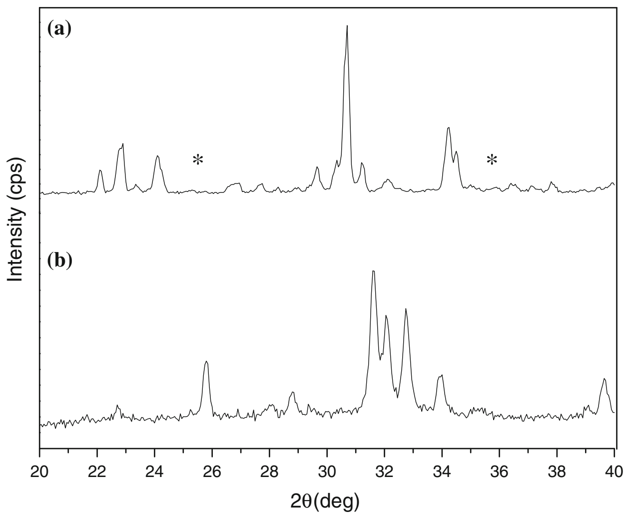

Fig. 1 XRD of *a* α-TCP (*β-TCP) and *b* hydroxyapatite whiskers

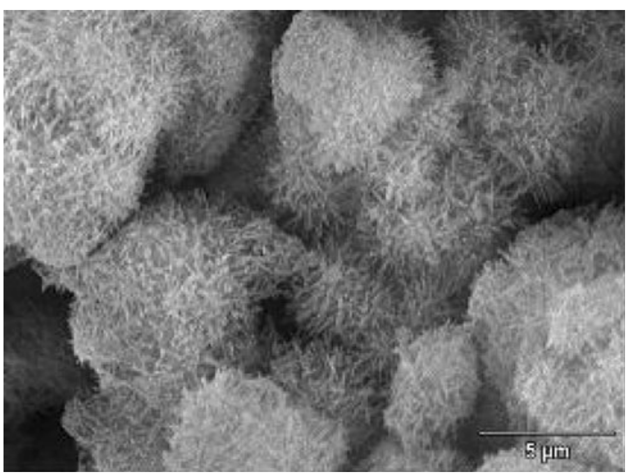

Fig. 2 SEM images of hydroxyapatite whiskers

like materials, and it was difficult to distinguish the individual fiber crystal. Nevertheless, the isolated hydroxyapatite whisker showed rod-like morphology, as can be seen in Fig. 2.

The X-ray diffraction was realized for samples of pure PCL and samples after the composite processing. The Fig. 3 shows the polymer crystallinity for pure samples and a different XRD spectrum for the composite samples. This difference is clearly due to the addition of the ceramic portion. Since the polymer peaks are steady in the same angles for both types of samples, it can be determined that the process does not influence in the final characteristics of the polymer.

Porosity was generated by mixing porogen salt particles with the PCL composite by a leaching technique. This is the easiest way of pore generation, and porosity degree and pores dimension can be tailored by opportune choice of particle size and concentration of the porogen.

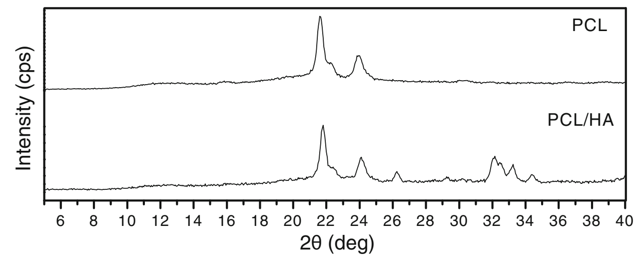

Fig. 3 XRD of PCL and PCL/HA

The porous morphologies resulting by salt leaching are clearly shown in the SEM micrograph, which reveals a quite homogenous structure, and well interconnected pores, in the Fig. 4a. No traces of remaining porogen can be seen. Unfortunately, problems with the dispersion of the whiskers inside the matrix resulted in a ceramic agglomeration, showed in Fig. 4b. This agglomeration problem is a current challenge in the production of PCL composites with nanofillers, possibly due to their great surface energy [9].

Well interconnected structures, as those shown by these scaffolds, are indispensable in tissue engineering issues, because they allow the migration of cells and body fluids into the scaffold, permitting a good diffusion of nutrients and eventually signaling substances throughout the structure, which represents a basic condition for tissue regeneration. Pore-throat size is related with the permeability and diffusivity throughout the material, and thus with the survival of implanted cells. The most porous structures are, the most likely to offer good conditions for homogeneous cell proliferation, because of their greater specific surface area.

Materials intended to be used for tissue engineering applications should not release any agent that may be cytotoxic to the human body. In order to evaluate if the composite at hand produces such toxic response, a test of cytotoxicity was performed. The result of this study revealed that no release of cytotoxic compound occurs from the prepared composite, Fig. 5.

Conclusions

This paper showed the methodology to prepare potential scaffolds of polycaprolactone/hydroxyapatite whiskers composites. The samples were analyzed by XRD, SEM, and EDS, and were possible to see structures with uniform porous morphology obtained by the salt leaching technique. A minor setback was observed in the process of mixing the filler portion in the polymeric matrix, resulting in non-homogeneous distribution. Biological evaluation evidenced complete absence of cytotoxicity in the composites. These

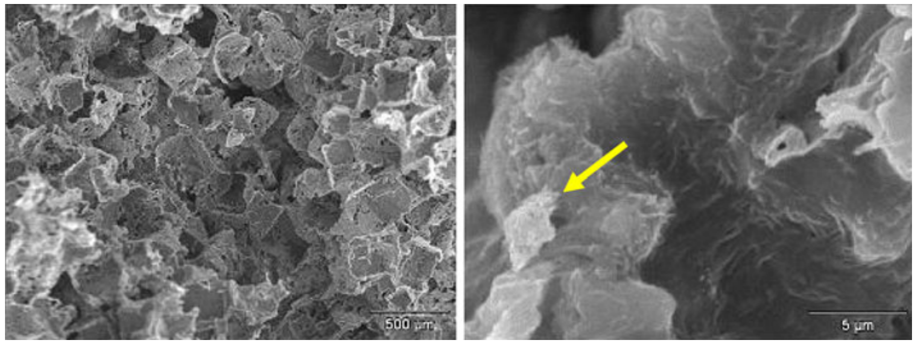

Fig. 4 SEM images of PCL/HA scaffolds: **a** clear pore production, **b** arrow showing whiskers penetration

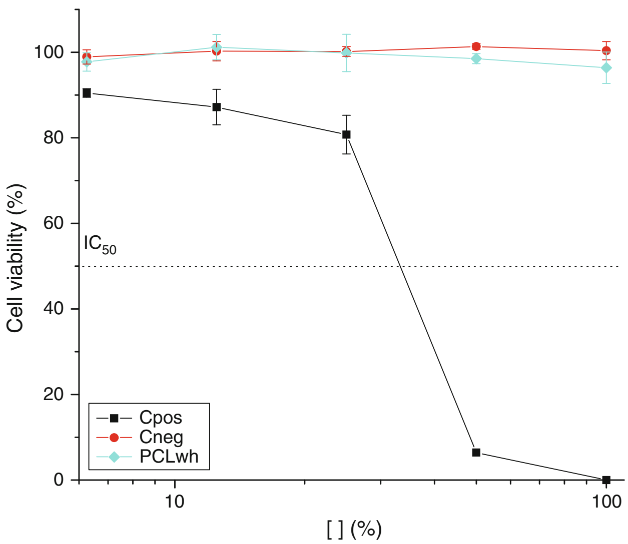

Fig. 5 Cytotoxicity test of hydroxyapatite whiskers

results suggest that this procedure to produce PCL/HA can be useful for the preparation of porous scaffolds for bone tissue engineering.

References

1. Amass W, Amass A, Tigue B (1998) Polym Int 47:89
2. Sabir MI, Xu X, Li L (2009) J Mater Sci 44:5713. doi: 10.1007/s10853-009-3770-7
3. Hao J et al (2003) Biomaterials 24:1531
4. Choi D, Marra KG, Kumta PN (2004) Mater Res Bull 39:417
5. Todo M et al (2009) J Mater Sci 44(15):4191. doi:10.1007/s10853-009-3546-0
6. Motisuke M, Carrodeguas RG, Zavaglia CAC (2008) Eng Mater 361–363:199
7. Ramos SLF, Motisuke M, Zavaglia CAC (2008) Eng Mater 396–398:501
8. Mikos AG et al (1996) Polymer membranes and methods of preparation of three dimensional membrane structures. US Patent, 5514378 7 de Maio de 1996
9. Deng C et al (2009) J Mater Sci 44(16):4394. doi:10.1007/s10853-009-3662-x