Development and cell response of a new biodegradable composite scaffold for guided bone regeneration

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Composites of biodegradable polymers with different calcium phosphate ceramics and glasses, have been developed as scaffolds for applications in bone–tissue engineering. In this work, phosphate glass particles have been incorporated into the polymer, poly(95L/5DL) lactic acid (PLA) and porous structures were elaborated. Their porosity, compressive mechanical properties and biological response were evaluated. Interconnected structures with evenly distributed pores and a porosity as high as 97% were obtained. The incorporation of glass particles into the polymer showed to have a positive effect in the mechanical properties of the foams. Indeed, the compressive modulus increased from 74.5 to 120 KPa and the compressive strength from 17.5 to 20.1 KPa for the PLA and the PLA/glass foams, respectively. The biological response was evaluated by means of the MTT test, the materials resulted to be noncytotoxic.

Introduction

Several techniques such as the utilisation of synthetic bone substitutes, autologous bone and allogenic bone for the restoration of bone injuries have been proposed along the last decades [1–4]. However, in general, some of these are not optimum. Besides, in the case of autografts, allografts or xenografts there are some well-known restrictions associated with donor site scarcity, rejection, diseases transfer and elevated harvesting costs.

These numerous drawbacks have led researchers to focus on the development of new possibilities such as tissue engineering. Nowadays, tissue engineering offers a promising approach to the repair of the bony tissue by using a porous scaffold that acts as a template for tissue regeneration. In this context, the development of different biodegradable porous scaffolds plays an important role. Lately, poly(α-hydroxyl acids) have been studied as materials for the elaboration of 3-D scaffolds for applications in tissue engineering given that they possess some desirable features as biocompatibility, bioresorbability, their degradation products are nontoxic and easily excreted by metabolic pathways and, besides, they are FDA approved for various applications [5].

Some calcium phosphate ceramics and biological glasses have been used for bone repair and reinforcement [6–8]. Specifically, calcium phosphate glasses, are well suited for bone repair given that they have a chemical composition close to that of the mineral bone phase, and their degradation rate can be controlled by modifying their chemical formulation [9]. Phosphate glasses in the system P_2O_5–CaO–Na_2O–TiO_2 have been studied in a previous work and have shown to be degradable at a controlled rate [10]. Besides, they have demonstrated to be noncytotoxic [11]. The aim of this work is to develop a biodegradable porous composite scaffold of poly(L/DL) lactic acid (PLA) and calcium phosphate glass in the system mentioned before, by combining the advantages of the two phases. The incorporation of phosphate glass particles has two purposes, the first one is to act as a mechanical reinforcement and the second purpose is to improve the cell–material interaction. The 3-D constructs are characterised in terms of their porosity, compressive mechanical properties and biological response.

Materials and methods

Materials

Poly(95L/5DL) lactic acid with an inherent viscosity of approximately 6.15 dl/g (PURAC, Holland) was used. Calcium phosphate glass particles with the following molar composition: 44.5P_2O_5–44.5CaO–6Na_2–5TiO_2

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were used to reinforce the polymer. For the elaboration of the glass $CaCO_3$, $NH_4H_2PO_4$, $NaCO_3$ and TiO_2 were used as raw materials. Briefly, the glass was melted in a platinum crucible at 1350 °C for 3 h in air, and then rapidly quenched on a metallic plate preheated at 350 °C. Finally, the glass was pulverised ($< 80\,\mu m$) with an agate planetary mill. Chloroform was also used as the polymer solvent and NaCl particles (250–500 μm) as porogenic agent.

Preparation of the polymer–glass composite foam

PLA was weighed accurately into a flask, then the precise amount of chloroform was added into the glass flask in order to obtain a 5% solution (w/v). The polymer–chloroform mixture was stirred at 25 °C until total dissolution of the polymer (approx. two days). Materials containing 0 and 40% (w/w) of glass particles were prepared. Thus, in the case of the PLA/glass composite, the glass particles were added to the polymer solution and mixed to make an homogeneous solution. For the fabrication of the foams, NaCl particles (94% w/w) were incorporated into the composite solution. Once mixed homogeneously, the slurry was cast in a teflon plate and allowed to remove any residual solvent. Foam discs were cut (20 mm diameter, 13 mm thick) from the foam plates.

The PLA–glass–salt composite specimens were removed from the moulds and immersed in a container filled with distilled water to follow a salt-leaching process and create the interconnected porosity. The container with the foams was kept under agitation to facilitate the salt-leaching. The foams were immersed for two days and the water changed four times per day. The efficiency of this protocol to remove totally the NaCl was assessed by SEM. After that, the foams were retrieved from the water and placed on blotting paper to drain. Finally, the foam samples were put in a furnace at 40 °C for 24 h to evaporate the residual water and were stored in a dessicator until characterisation.

Characterisation of the foams

The porosity of the PLA and PLA/glass foams were quantified using an indirect method based on the Archimedes' Principle. The apparent density (ρ_a) of the foam was obtained by mercury immersion. Thus, using the apparent densities and the densities of the nonporous PLA (ρ) and composite (measured by picnometry), the porosity of the foams was determined using the following equation [12]:

$$P = 1 - \frac{\rho_a}{\rho} \qquad (1)$$

where ρ_a is the apparent density and ρ is the density of the non-porous material.

The compressive mechanical properties of the 3-D constructs were measured using an electromechanical universal testing machine (MTS-ADAMEL) equipped with a 100 N load cell. Composite and PLA foam discs (20 mm diameter, 13 mm thick) were used. A crosshead speed of 2 mm/min was used for evaluating the compressive properties of the foams. The load was applied until the foam was compressed to 50% of its original thickness. The compressive modulus was defined as the initial linear modulus while the yield strength was determined from the cross point of the two tangents on the stress–strain curve around the yield point [13]. Five specimens of each sample were tested.

Scanning electron microscopy (SEM) was performed to observe the morphology of the foams as well as the distribution and interface of the glass particles into the polymer matrix. To this end, a gold-coated section of each type of foam was observed.

Cytotoxicity test

For the cell cultures, SAOS-2 osteoblast-like cells originally isolated from a human osteogenic osteosarcoma, were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum, 1% penicillin/streptomycin and 2 mM L-glutamine at 37 °C in a humidified atmosphere of 5% CO_2 in air. The cultured media was changed every two days and for subculture the cells monolayers were rinsed with phosphate-buffered saline (PBS) and detached by incubating them with Trysin–EDTA (0.25%) for 5 min at 37 °C. The detached cells were recultured following the conditions for the toxicity test.

A concentration of 7×10^4 cells/well was cultured on the PLA and PLA/glass scaffolds previously sterilised by ethylene oxide. Located in a 24-well plate with 50 μl of DMEM per well for the evaluation of the cytotoxicity in direct contact with the cells. The cells were allowed to attach for 30 min to the substrates, then 500 μl of the culture medium were added to each well. The polysterene standard culture plate was used as control.

After 24 h, three and six days, 1 ml of MTT solution (10% in culture medium without phenol red) was added to each well in order to measure the mitochondrial activity of the cells. Absorbance was measured after 3 h of contact with the MTT salt at 570 nm using an HP8453 spectrophotometer. The results are expressed as the average absorbance values of three replicates. A one-way ANOVA test was performed to determine the statistical significance $(p < 0.05)$ of the differences in the absorbance values.

Results

Using the processing technique previously described, both PLA and PLA/phosphate foams, were manufactured using only biodegradable materials. SEM images (Fig. 1) showed that highly porous and interconnected structures were obtained. The structure of the PLA foams and pore morphology was very similar to that of the porous composite. Besides, a good adhesion and distribution of the glass particles in the polymeric matrix were also observed. Indeed, Fig. 1(b) shows the polymer film covering one of the glass particles.

According to the method used for the determination of the porosity, the material made of PLA presented 94% of porosity while the material elaborated with the PLA/glass blend reached a 97% of porosity.

The compressive modulus and yield strength were measured for the PLA template and the composite foam

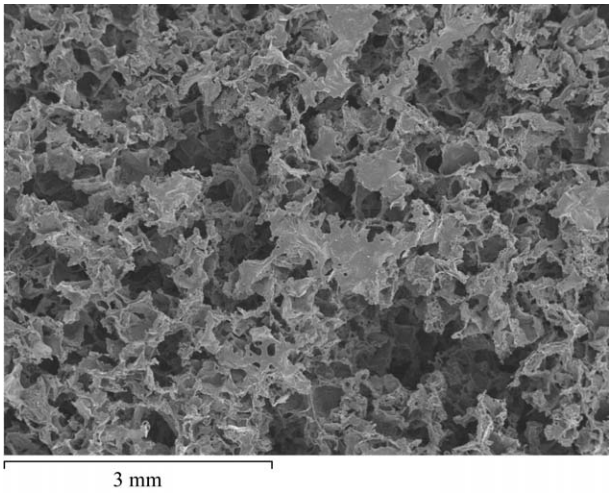

Figure 1 SEM micrographs of the fracture surface of PLA and PLA/glass foams. (a) PLA foam; (b) glass particle covered with PLA.

with 40% by weight calcium phosphate glass particles. The results are displayed in Table I. As can be observed, both the compressive yield strength and the modulus increased significantly with the addition of the glass particles as reinforcement. The compressive modulus increased up to 120 ± 3.1 MPa for the PLA/glass foam. In comparison, the modulus obtained for the PLA foam was 74.5 ± 1.5 MPa. Respecting the compressive yield strength, the addition of the glass particles into the polymeric foams increased the values from 17.5 ± 0.92 to 20.2 ± 1.05 KPa.

According to Fig. 2, a noncytotoxic effect could be noticed for the two foams, PLA and PLA/glass. Even though after 24 h of culture the cell response was better for the control plate than for the studied materials, it could be observed that after three and six days of culture, the absorbance values resulted to be significantly higher both, for the polymeric and the composite foams than for

TABLE I Porosity percentage and compressive mechanical properties of the studied foams (value ± SD)

Foam	%Porosity	*E*(KPa)	σ(KPa)
PLA	93 ± 1.25	74.5 ± 0.15	17.5 ± 0.92
PLA/glass	97 ± 0.51	120 ± 0.03	20.1 ± 1.05

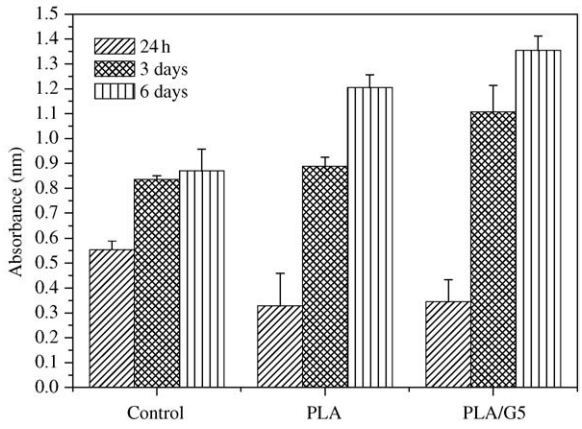

Figure 2 MTT assay of the effect of the PLA and PLA/glass foams in direct contact with osteoblastic cells after 24 h, three and six days of culture. Vertical lines represent ± SD.

the control. In addition, the composite formed by the PLA/glass combination, presented statistically significant higher absorbance values ($p < 0.05$) than the PLA foams.

Discussion

Highly porous materials with a high level of interconnectivity are required for the elaboration of scaffolds for guided bone regeneration. The foams obtained in this work showed to have a porosity as high as 97% and also a good interconnectivity as could be observed in the SEM images. The level of interconnectivity is of great importance since the tissue ingrowth and vascularisation as well as the delivery of nutrients throughout the newly formed tissue depends strongly on this parameter. On the other hand, the NaCl particles used as porogen had a size between 250–500 µm, thus the obtained porosity resulted to be in a range appropriate for bone tissue ingrowth into the biodegradable foams [14–16].

The incorporation of calcium phosphate glass particles to the PLA foams improved both, the mechanical properties and the biological response of the scaffolds. The values obtained for the compressive modulus showed to be in agreement with the values obtained in other studies for materials with similar porosities [17, 18]. The results showed that the compressive mechanical properties improved with the addition of the glass, thus the particles fulfilled their reinforcing role. When the glass particles are introduced into the 3-D structure of the polymer foam, the pore walls get more rigid and as a consequence, they do not bend and collapse as easily as in the case of the polymer foams. Besides, the fact that the compressive properties have increased, indicates a good initial adhesion between the PLA and the reinforcement.

Even though the test performed in this study does not allow a deep analysis of the biological interaction between the cells and the material, it allows the evaluation of the initial cell response when the cells are in contact with it, which is of great importance for the study of the biocompatibility.

According to the obtained results (Fig. 2), the cell viability increased with the culture time for the three different materials, the control polysterene, the PLA

foam and the PLA/glass foam, indicating that cells proliferate adequately. However, the increment in the absorbance values is more evident in the case of the foams. Moreover, the PLA/glass foam showed higher values than the PLA foam. Thus, these results suggest that the glass particles had a positive influence in the cell viability and proliferation.

Conclusions

A new highly porous composite formed by PLA and a soluble calcium phosphate glass has been developed for bone–tissue engineering applications. An interconnected structure with a porosity as high as 97% was obtained. The resulting data indicated that glass particles enhanced both, the compressive properties and the biological response of the foams. The optimisation of the composite should allow to better control the scaffold performance.

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Received 4 October
and accepted 10 October 2003