

The synergic effect of polylactide fiber and calcium phosphate particle reinforcement in poly ϵ -caprolactone-based composite scaffolds

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Abstract

In this work, three-dimensional porous composite scaffolds, based on poly(ϵ -caprolactone) (PCL), were fabricated through the combination of a filament winding technique and a phase inversion/salt leaching process. Sodium chloride crystals were used as the porogen agent, and poly(lactic acid) (PLA) fibers and calcium phosphates as reinforcement. The aim of the current work is to assess the effective synergistic role of bioactive particles (i.e. α -tricalcium phosphates (α -TCP)) and PLA fibers on the morphology and mechanical response of the final scaffold. Morphological investigations performed on fiber-reinforced composite scaffolds with different PCL/ α -TCP volume ratios (0%, 13%, 20% and 26%) show a high porosity degree (ca. 80%), pore interconnection and a homogeneous distribution of pores within the scaffold. More specifically, a bimodal pore size distribution was observed. This comprised microporosity (pores with radii ranging from 0.1 to 10 μm , which were strictly related to solvent extraction) and macroporosity (pores with radii from 10 to 300 μm , which were ascribable to the leaching of porogen elements). Static compressive tests showed that the effect of α -TCP on the mechanical response was to increase the elastic modulus up to a maximum value of 2.21 ± 0.24 MPa, depending on the concentration of α -TCP added. This effect may be explained through the interaction of calcium-deficient hydroxyapatite crystals, formed as a consequence of a hydrolysis reaction of α -TCP, and the fiber-reinforced polymer matrix. The correct balance between chemical composition and spatial organization of reinforcement systems allows the attainment of an ideal compromise between mechanical response and bioactive potential, facilitating the development of composite scaffolds for bone tissue engineering applications.

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1. Introduction

Skeletal tissues, such as bone and cartilage, may be considered as three-dimensional (3-D) composite systems organized by hierarchical structures, the properties of which vary as a function of the dimensional scale. The high complexity degree of intrinsic properties of the natural tissues imposes the need to define appropriate strategies for the design of 3-D scaffolds with tailored properties, adapted to be effective in tissue engineering applications [1,2].

High porosity and pore interconnectivity are key requisites to increase the specific surface area available for cell

attachment and tissue in-growth, so facilitating the uniform distribution of cells and the adequate transport of nutrients and cellular waste products. Taking into account the intimate correlation between specific cells and pore sizes for optimal cell attachment and growth [3], it is crucial to develop polymeric scaffolds with a high degree of porosity but, simultaneously, with good control over the pore size and morphology [4,5].

Scaffold design may be more complex in the case of the repair of hard tissues such as bone. Bone tissue is organized into microstructural composites based on the interdigitation of collagen – the most prevalent biopolymer in the body – and an inorganic substance composed of apatitic mineral crystals. The primary function of the latter is to support loads adequately [6]. In this case, the scaffold

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should not only be a highly porous structure, able to guide novel tissue formation in 3-D space, but also it should have sufficient mechanical strength to offer adequate structural support. Furthermore, it should be able to stimulate new bone growth, resulting, finally, in native bone tissue with no trace of the scaffold.

To date, the employment of composite materials may be a good solution, achieving the ideal balance between strength and toughness due to the improvement of specific characteristics of the composite compared to its separate components [7]. However, the need of scaffold materials to be porous, biocompatible and biodegradable, and to exhibit a degradation or resorption rate similar to the rate of tissue replacement, is often in conflict with the possession of adequate mechanical properties able to match those of the tissues at the site of implantation [5]. In this context, the appropriate selection of materials may be crucial to define the basic properties of the scaffold, which will determine, to a great extent, the ultimate properties of the newly formed engineered tissue [8]. More specifically, the definition of composite systems characterized by tailored degradation properties, coupled with a controlled decay of mechanical response, may guarantee the achievement of the long-term success of a tissue engineered construct [8,9].

The current approach lies in the development of composite materials which comprise a biodegradable matrix incorporating bioactive rigid particles, which combine the reinforcement action of hydroxyapatite (HA)-based particles with the tailored degradation kinetics of resorbable polymers [10,11].

To date, several studies have examined the use of bio-ceramic particles such as silica, HA or other calcium phosphates in combination with biodegradable polymers (i.e. poly(ϵ -caprolactone) (PCL), poly(lactic acid) (PLA)) to produce bone substitutes. This is because of the structural similarity poly(lactic acid) (PLA) of these systems to the mineral phase of bone, and to their osteoconductive and bone binding properties [12–15]. However, most results taken from previous studies reveal that, while the incorporation of a ceramic phase improved the bioactivity of the polymeric scaffold, this advantage is not usually combined with a commensurate enhancement of the mechanical properties of the composite [16]. Other authors have described the limited reinforcement offered by HA micrometric particles within a PCL matrix, indicated by particle overexposure on the pore surfaces, combined with a tendency to form clusters [17].

Recently, Ambrosio et al. [18] proposed an alternative composite tubular structure. This is obtained by the merging of a polyurethane matrix with continuous fibers of PLA and poly(glycolic acid) (PGA) helically wound by the filament winding technique. By applying the basic theory of continuous fiber-reinforced composites to the scaffold design, a composite material has been developed with an optimal spatial organization of fibers within the polymer matrix, able to mimic the structural organization and performance of the living tissue.

In this work, a novel composite scaffold was proposed which combines the use of two reinforcement systems in different forms – particles and long fibers – to optimize the final mechanical response of the scaffold. Here, three-dimensional porous PCL-based composite scaffolds, tubular in shape, were prepared by the combination of the filament winding technique and a phase inversion/salt leaching process. The employment of highly biocompatible and bioresorbable PCL and PLA assures the maintenance of sufficient physical and mechanical properties for at least six months before their degradation [8]. The integration of a solid porogen (i.e. sodium chloride crystals) within a 3-D polymer matrix enables creation of an interconnected pore network with well-defined pore sizes and shapes. Tri-calcium phosphate (α -TCP) powder, which is able to precipitate in calcium-deficient hydroxyapatite (CDHA) form, is recognized by the host tissue as being similar to natural bone apatite [19].

In this work, we have investigated the synergistic contribution to the mechanical response of a scaffold oriented to mimic bone functional behavior by the interaction between ceramic phase and a highly organized, continuous fiber network.

2. Materials and methods

2.1. Scaffold preparation

The preparation of the PCL composite scaffolds is graphically described in Fig. 1. Firstly, a polymer solution was prepared by dissolving PCL pellets (mol. wt. 65 kDa; Sigma Aldrich) in *N,N*-dimethylacetamide (J.T. Baker), at 20 wt.%, at 58 °C for about 3 h under stirring. Afterwards, NaCl particles sieved into a specific size range (300–500 μ m) were added (salt/polymer weight ratio of 9/1) to form a homogeneous mix. Meanwhile, a mixture of calcium phosphate powders called Hcem (98 wt.% α -TCP, 2% HA – kindly supplied by Universitat Polytechnica de Catalunya, Barcelona, Spain) was introduced into the polymer and salt solution at different volume ratios (13, 20 and 26 vol.%).

A fiber-reinforced composite structure was obtained by a conventional technique of filament winding: PLA fibers (75 dtex), impregnated in the previously described polymeric solution, were wound helically by a winding machine using a winding angle of 45° on a Teflon-coated steel mandrel of 1 mm diameter. Each specimen was prepared using an equal PLA/PCL weight ratio, defined by the fiber-cycle number counted during the winding process. Finally, ethanol (C₂H₅ OH) was used for 24 h to extract the solvent from the composite scaffold which was then totally immersed in distilled water for seven days to enable the salt crystals and any other contaminants to leach out.

2.2. Prediction of fiber/matrix ratio

In order to establish the final composition of the scaffold, the exact amount of PLA fibers with respect to the

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