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Composite fibrous biomaterials for tissue engineering obtained using a supercritical CO_2 antisolvent process

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Abstract

Several techniques have been proposed for producing porous structures or scaffolds for tissue engineering but, as yet, with no optimal solution. With regard to this topic, this paper focuses on the preparation of biocompatible nanometric filler–polymer composites organized in a network of fibers. Titanium dioxide (TiO_2) or hydroxyapatite (HAP) nanopowders as the guest particles and poly(lactic acid) (L-PLA) or the blend poly(methylmethacrylate)/poly(ε-caprolactone) (PMMA/PCL) as the polymer carrier were selected as model systems for this purpose. A supercritical antisolvent technique was used to produce the composites. In the process developed, the non-soluble particulate filler was suspended in a polymer solution, and both components were sprayed simultaneously into supercritical carbon dioxide ($scCO_2$). Using this technique, polymeric matrices were loaded with ~10–20 wt.% of inorganic phase distributed throughout the composite. Two different hybrid materials were prepared: a PMMA/PCL + TiO_2 system where either fibers or microparticles were prepared by varying the molecular weight of the used PMMA; and fibers in the case of L-PLA + HAP system. After further post-processing in a three-dimensional network, these nanofibers can potentially be used as scaffolds for tissue engineering.

Keywords: Supercritical CO_2; Nanocomposite; Fibrous tissue; Scaffold; Bone tissue engineering

1. Introduction

Tissue engineering is based on tissue formation and regeneration using artificial scaffolds specifically designed to direct tissue growth. Ceramics, polymers and composites have traditionally been used in tissue repair, polymers being the primary materials used for scaffolds [1,2]. Collagen, gelatin, poly(lactic acid) (PLA), poly(lactic-co-glycolic acid) and poly(ε-caprolactone) (PCL) are a few notable examples of biodegradable polymers used. Poly(methylmethacrylate) (PMMA), poly(ethylene), poly(vinyl alcohol) and polycarbonate are examples of non-biodegradable polymers. Not only the material but also the architecture of the scaffold plays an important role in modulating the tissue growth and response behavior of cultured cells [3,4]. Laboratory-designed scaffolds adopt forms ranging from monolithic microcellular structures (sponges) to networks of fibers [4,5]. The use of polymer fibers seems to have some intrinsic advantages from a biomimetic approach [6–8].

Among the most widespread fibrous scaffold fabrication processes, rapid prototyping techniques (f.i., three-dimensional (3D) plotting or 3D fiber deposition) produce micrometric fibers (100–300 μm) that form networks characterized by fully interconnected pores with diameters in the order of 200–1000 μm [9,10]. However, the importance of an internal architecture that mimics the nanofibrous structure of the natural extracellular matrix is nowadays highly recognized [11]. In this respect, electrospinning technology

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has been used for the fabrication of non-woven nanofibrous scaffolds [12–14]. However, the fabricated scaffolds generally lack the necessary biomechanical properties. To improve the structural properties, post-processing treatments, such as fiber bonding techniques have been developed [11]; for example, interconnected fiber networks have been prepared by thermal treatment [15–17]. Moreover, dual scale scaffolds have been obtained using hybrid processes that inserted electrospun nanofibers in the micropores of a 3D scaffold obtained using a rapid prototyping technique [18].

This work examines the feasibility of a supercritical fluid assisted technique for the formation of nanofibers. The network of fibers obtained was morphologically different from that formed using electrospinning methods: whereas electrospinning methods produce only one very long fiber that is further networked on a surface, a disordered network of relatively short fibers was created using the supercritical approach. Technology based on supercritical carbon dioxide ($scCO_2$) has been established as an alternative to overcome some of the problems associated with the both the use of organic solvents and high temperatures for biomaterial preparation [19–25]. The absence of solvents and thermal processing makes it an attractive approach for preparing biomaterials that incorporate drugs or proteins [26,27]. $scCO_2$ is widely used to produce polymer sponges by pressure-induced phase separation [25,28–30]. Supercritical antisolvent spray processes have also achieved considerable success in producing polymers with different morphologies and composite microspheres by coprecipitation [31–39].

Previously, experiments were carried out based on the precipitation of fibrous matrices consisting of the biodegradable L-PLA or the blend PMMA/PCL using the antisolvent $scCO_2$ technique [40]. Blending of polymers is considered to be an efficient approach to the preparation of materials with new properties. In particular, the blend of PCL and PMMA appears to provide materials with good mechanical integrity and biodegradation capabilities [41]. Furthermore, nanostructured hybrid organic/inorganic composites have attracted considerable attention in many areas of polymer applications. The introduction of small quantities of nanosized inorganic particles can significantly improve the mechanical and physical properties of the polymer matrix [42–45]. The scaffold can also be formulated to contain additives or active agents to allow fast tissue growth. For example, nanohydroxyapatite (HAP) based composites have gained much recognition as bioactive bone grafts, owing to their compositional and structural similarity to natural bone [3,46,47]. The main aim of this work was to demonstrate that fibers containing ultrafine mineral particles can also be prepared using the antisolvent $scCO_2$ technique. Moreover, for *in vivo* applications, the inflammatory response in the immediate post-transplant period can be circumvented using fibrous scaffolds releasing non-steroidal anti-inflammatory drugs [48]. To this end, the encapsulation of the anti-inflammatory drug ketoprofen was also attempted.

2. Materials and methods

2.1. Materials

The characteristics of the polymers used are shown in Table 1. $Blend_1$ (B_1) and $Blend_2$ (B_2) were composed of $PMMA_1$/PCL and $PMMA_2$/PCL, respectively. To evaluate the efficiency of the $scCO_2$ encapsulation process, nanoparticles of titanium dioxide (TiO_2) were first chosen as the model inorganic filler. The nanometric powder (P-25S, Degussa) was silanized with octyltriethoxysilane using a $scCO_2$ technique (samples labeled T_sSC). A detailed description of the silanization process can be found in the literature [49]. Degussa silanized TiO_2 (T805 that corresponds to the P-25S treated on the surface with octyltrimethoxysilane) was used for comparison (samples labeled T_sC). Next, nanometric HAP was used as a filler. The HAP used was synthesized in the authors' laboratory following a procedure described elsewhere [50] and silanized with γ-methacryloxypropyltrimethoxysilane in $scCO_2$ [49] (samples labeled H_sSC). Ketoprofen (Aldrich) was used as the model drug (samples labeled with a K). Dichloromethane (DCM, Prolabo) and CO_2 (99.95 wt.%, Air Liquide) were the fluids used.

2.2. Methods

Experiments were carried out in a PCA (particles from a compressed antisolvent) apparatus shown schematically in Fig. 1, operated in batch mode. The experimental set-up consisted of a CO_2 supply line (CO_2 in), a solution delivery line (solution in) and a high-pressure vessel with a capacity of 0.5 L (Re1, Autoclave Engineers). The vessel temperature was controlled by heating jackets. At the bottom of the vessel, a membrane filter placed on the top of a stainless steel frit of porosity 2 μm allowed the collection of precipitated solids. The CO_2 was first cooled (EX1) and then pumped using a reciprocating pump (P1, Lewa EK3). The liquid solution was compressed using a dual-piston minipump (P2, Milton Roy CP300). The pressure inside the vessel was controlled downstream with a micrometering valve (V5). Blend solutions were prepared by adding 15.6 and 3.2 mg of PMMA and PCL, respectively, to each

Table 1
Characteristics of employed raw polymers (χ = crystallinity).

Polymer	Supplier	Molecular weight ($g\ mol^{-1}$)	Structure	Thermal transitions (°C)
$PMMA_1$	Bonar polymers (Colacryl 300)	300,000	Amorphous	$T_g = 120$
$PMMA_2$		120,000	Amorphous	$T_g = 119$
PCL	Aldrich	14,000	Semicrystalline, $\chi = 68\%$	$T_m = 67$
L-PLA	Biovalley	100,000	Semicrystalline, $\chi = 50\%$	$T_g = 47$ $T_m = 176$

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