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New porous polycaprolactone–silica composites for bone regeneration

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ABSTRACT

Polycaprolactone porous membranes were obtained by freeze extraction of dioxane from polycaprolactone–dioxane solid solutions. Porosities as high as 90% with interconnected structures were obtained by this technique. A silica phase was synthesized inside the pores of the polymer membrane by sol–gel reaction using tetraethylorthosilicate (TEOS) as a silica precursor and catalyzed in acidic and basic conditions. Two different morphologies of the inorganic phase were obtained depending on the type of catalyst. In acid catalyzed sol–gel reaction, a homogeneous layer of silica was deposited on the pores, and discrete microspheres were synthesized on the pore walls when a basic catalyst was used. The morphology of the inorganic phase influenced the mechanical and thermal behavior, as well as the hydrophilic character of the composites. Bioactivity of the porous materials was tested *in vitro* by measuring the deposition of hydroxyapatite on the surfaces of the porous composite membranes. Polycaprolactone/silica composites revealed a superior bioactivity performance compared with that of the pure polymer; evidenced by the characteristic *cauliflower* structures on the material surface, increase in weight and Ca/P ratio of the hydroxyapatite layer. Also, the acid catalyzed composites presented better bioactivity than the base catalyzed composites, evidencing the importance in the morphology of the silica phase.

1. Introduction

PCL is a linear aliphatic, hydrophobic and semicrystalline polyester. PCL has gained an increased interest in the biomedical field due to its good solubility, satisfactory mechanical properties, low melting point (59–64 °C) and easy shaping and manufacture. In particular, PCL has been proposed as a support material in bone tissue engineering [1–3]. In that kind of application, PCL scaffolds have been produced by a variety of techniques, including rapid prototyping [2–6], the use of templates [7], freeze extraction [8,9] and casting [10].

PCL hydrolytic degradation may be considered a slow reaction, however, enzymatic degradation mechanisms can contribute to in vivo bioresorption of PCL implants [6,11]. Also, the reported elastic modulus of PCL appears to be low for some applications in tissue engineering (ranging from ~10 up to 280 MPa) [12–14], being considerably smaller (between 0.5 and 8 MPa) for porous membranes [8,15,16].

Many polymer composites based on PCL have been developed in order to increase stiffness and improve their bioactivity by the incorporation of fillers as nano or micro particles of tricalcium phosphate (TCP) [17], mesoporous wollastonite particles [18,19], bioactive glass as 45S5 Bioglass® (BG) [20,21], hydroxyapatite [22,23], and mesoporous silicate [24]. Diverse methods such as solvent casting, solid–liquid phase separation or melt mixing have been used for dispersing the fillers in the PCL matrix. The biological response of the composites has been evaluated in bone tissue engineering, showing no cytotoxicity and on the contrary, a better performance to cell adhesion. It has been found that the osteoblastic differentiation is a function of the amount of filler and the spatial distribution of the particles in the composite [21,23,25–29]. In this vein, to improve bioactivity, several approaches have been done to cover the macropores of polymeric scaffolds with a hydroxyapatite layer by immersing the porous materials in simulated body fluids [16,30,31].

Much interest has been paid to both biological silicon-based metabolic pathways and the role of silicon in osteogenesis, atherosclerosis and biogenesis. Reports of silica (SiO_2), as a biocompatible and bioactive material, have stimulated interest in the biological properties of this ceramic for bone tissue applications, such as rate of bioresorption and

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porosity, as well as the ability to induce formation of mineral calcium phosphate on surfaces similar to that of bone, and the introduction of biological active agents [13,32,33]. Since silica reinforcements are used frequently in the form of nanoparticles, understanding of mechanisms of dissolution and metabolism of these particles in the organisms has also deserved considerable study [32].

Sol–gel reaction, is a method that has been used to produce silica coatings on metal [34] and polymer surfaces [35]. Lately, this technique has become widely spread in biomaterial synthesis due to its simplicity, low cost and efficiency for producing high-quality surfaces [36]. The sol–gel reactions involve a series of steps that could be modified to induce specific properties on the material. First, a liquid alkoxide precursor, such as $Si(OR)_4$ being $R = C_2H_5$ in the case of tetraethylortosilicate (TEOS), is hydrolyzed by mixing it with water as shown in Reaction 1.

$$Si(OC_2H_5)_4 + 4H_2O \rightarrow Si(OH)_4 + 4C_2H_5OH \quad \text{Reaction1}$$

Then, fully hydrolyzed $Si(OR)_4$, interacts by a condensation reaction until forming

$\equiv Si-O-Si\equiv$ bonds (Reaction 2)

$$Si(OH)_4 + Si(OH)_4 \rightarrow (OH)_3Si-O-Si(OH)_3 + H_2O. \quad \text{Reaction2}$$

A SiO_2 network is the result of the association of additional tetrahedral $\equiv Si-OH$ groups. Particles act as a colloidal system (or a sol) when a sufficient number of interconnected $Si-O-Si$ bonds are formed. The size and crosslinking density of the sol particles depend on the pH, the ratio R ($R = [HO]/[Si(OR)_4]$), and other conditions such as the temperature, nature and concentration of electrolyte, nature of the solvent and type of alkoxide precursor [36–39].

This work was focused on obtaining porous membranes of PCL–silica nanocomposites by introducing a TEOS solution into the pore structure of a previously formed PCL membrane. Sol–gel reaction took place in the pores of the membrane using an acidic or a basic catalyst. Microstructure, physical properties and in vitro bioactivity of the produced composites were studied. The original PCL membrane has the microstructure of the pore walls of a previously developed macroporous scaffold aimed for bone regeneration [29]. The results of this study will allow in the future obtaining hybrid PCL-Si macroporous scaffolds.

2. Materials and methods

2.1. Materials

PCL pellets (Aldrich, $\overline{M}_w = 70{,}000–90{,}000$ (GPC)), 1,4-dioxane (DX) (Scharlab, reagent grade, stabilized with 2.5 ppm of BHT), ethanol absolute, tetraethylorthosilicate (Sigma-Aldrich, reagent grade 98%) and ammonium hydroxide (NH_4OH) (Scharlab, 25%) were used as received. Hydrochloric acid (HCl) (Scharlab, 37%), was diluted with purified water to obtain a 10% solution.

2.2. Preparation of PCL microporous membranes by freeze extraction

Solutions of PCL/DX with PCL concentrations of 5, 10, 15 and 20% (w/w) were stirred at room temperature until total dissolution. Then, solutions were poured into PTFE evaporating dishes to obtain membranes of ~2 mm thickness. The whole system was quickly immersed in liquid nitrogen until the solutions took an opaque white appearance, evidencing a two-phase system. Then, chilled ethanol ($T \sim -10$ °C) was transferred over the frozen PCL solution and stored at −20 ºC. Ethanol at $T < -10$ °C was changed several times to ensure that the DX phase was totally dissolved. The microporous membranes obtained were dried in vacuum at room temperature until constant weight (around 2 days). They were cut to have samples of appropriate size and shape for applying characterization techniques. Samples will be designated as PCLXX, where "XX" means the weight percentage of PCL in the PCL/DX solutions.

2.3. Introduction of the silica phase into the PCL porous membranes

Polymer/silica composites were prepared using TEOS as a silica precursor, and HCl (10% aqueous solution) and NH_4OH (25% w/w aqueous solution) as acidic and basic catalysts respectively. For acidic conditions, the molar ratio used was TEOS/water/ethanol/HCl 1/2/1/0.0185, while for basic conditions it was TEOS/water/ethanol/NH_4OH 1/2/1/0.138. For preparing TEOS acid solution, the calculated volumes of TEOS, ethanol and water, were added in this order and mixed by means of a constant stirring for 5 min, and then HCl 10% was added under stirring for an additional hour. For base catalyzed reaction, the calculated volumes of TEOS, ethanol and water, were added in sequence and stirred for 1 h. After adding the catalyst (NH_4OH, 25%), the mixture was subjected to an additional minute of stirring. To facilitate the penetration of TEOS solution into the pores of PCL membranes, high vacuum was applied for 5 min to a vacuum line to which a tube containing the PCL membrane samples was joined. The surface of the samples was washed with a mixture of ethanol–water (same ratio as used in the TEOS solution) and kept overnight (14–18 h) at room temperature and finally at 40 °C for 12 h. The samples were dried in vacuum for 24 h and weighed. Dry membranes were washed by immersion in water with continuous stirring from 2 to 5 h, dried overnight at room conditions and then in vacuum desiccator at room temperature for 2–3 days until constant weight. The samples were designated as PCLXXSiA or PCLXXSiB, where XX refers to the percentage of PCL in the DX solution, "A" means acid catalyzed and "B" means base catalyzed sol–gel reaction. The reproducibility of the characterization methods was tested, and most of the reported values correspond to the average of at least three different measurements.

2.2. Characterization of porous PCL and PCL/silica membranes

Average porosity (ϕ) was determined gravimetrically. Dry samples, previously weighed, were immersed in ethanol under vacuum. The excess of ethanol was wiped off by gently absorbing surface drops with a filter paper, and the weight was determined again. Porosity was calculated as the ratio of the volume of pores (V_{pores}) to total volume of the sample $V_{pores} + V_{mat}$, where V_{mat} is the volume of the membrane material. Assuming that the amount of ethanol absorbed by the bulk material was negligible, the porosity may be expressed as follows

$$\phi = \frac{V_{pores}}{V_{pores} + V_{mat}} = \frac{m_{et}/\rho_{et}}{m_{et}/\rho_{et} + m_{mat}/\rho_{mat}} \quad (1)$$

where, m_{et} is the mass of absorbed ethanol, m_{mat} is the mass of the dry membrane, ρ_{et} is the density of ethanol, and ρ_{mat} is the density of bulk material. For porous PCL $\rho_{mat} = \rho_{PCL} = 1.145$ g/cm^3[8]. In the case of the composites, the density of the bulk material (ρ_{hyb}) could be approximated by

$$\frac{1}{\rho_{hyb}} = \frac{1-w_{SiO_2}}{\rho_{PCL}} + \frac{w_{SiO_2}}{\rho_{SiO_2}} \quad (2)$$

w_{SiO_2} being the weight fraction of silica in the composite and $\rho_{SiO2} = 2.634$ g/cm^3. Silica content in the composites was determined by weighing dry samples before and after sol–gel reactions. Two additional methods were used to determine silica content: (*i*) pyrolysis treatment for 2 h using an electrical tubular oven (Gallur, Spain) at 850 ºC in oxygen atmosphere and (*ii*) measuring the inorganic residue produced after thermogravimetric analysis (TGA) (SDT Q600 analyzer, TA Instruments), determining the residue after a heating scan at 10 ºC/min (under nitrogen atmosphere) from room temperature up to 850 ºC.