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Tailoring the morphology of high molecular weight PLLA scaffolds through bioglass addition

N. Barroca [a], A.L. Daniel-da-Silva [b], P.M. Vilarinho [a], M.H.V. Fernandes [a,*]

[a] Department of Ceramics and Glass Engineering and Centre for Research in Ceramics and Composite Materials, CICECO, University of Aveiro, 3810-193 Aveiro, Portugal
[b] Department of Chemistry and Centre for Research in Ceramics and Composite Materials, CICECO, University of Aveiro, 3810-193 Aveiro, Portugal

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ABSTRACT

Thermally induced phase separation (TIPS) has proven to be a suitable method for the preparation of porous structures for tissue engineering applications, and particular attention has been paid to increasing the pore size without the use of possible toxic surfactants. Within this context, an alternative method to control the porosity of polymeric scaffolds via the combination with a bioglass is proposed in this work. The addition of a bioactive glass from the $3CaO{\cdot}P_2O_5$–MgO–SiO_2 system enables the porous structure of high molecular weight poly(L-lactic) acid (PLLA) scaffolds prepared by TIPS to be tailored. Bioglass acts as a nucleating catalyst agent of the PLLA matrix, promoting its crystallization, and the glass solubility controls the pore size. A significant increase in the pore size is observed as the bioglass content increases and scaffolds with large pore size (~150 μm) can be prepared. In addition, the bioactive character of the scaffolds is proved by in vitro tests in synthetic plasma. The importance of this approach resides on the combination of the ability to tailor the porosity of polymeric scaffolds via the tunable solubility of bioglasses, without the use of toxic surfactants, leading to a composite structure with suitable properties for bone tissue engineering applications.

1. Introduction

Nowadays therapeutic strategies based on tissue engineering for organ regeneration use three-dimensional (3D) scaffolds as synthetic extracellular matrices designed to allow cells to proliferate and secrete their own extracellular matrix while the scaffold gradually degrades [1]. Requirements for these 3D structures are manifold and depend on the application. For example, for bone tissue regeneration scaffolds should be biocompatible and should have adequate mechanical properties. Further requirements include controllable biodegradability, as the degradation and resorption rates should match cell/tissue growth, and also a pore size >100 μm and good pore interconnectivity to allow vascularization and tissue ingrowth [2].

Frequently these 3D structures consist of polymer/ceramic composites, such as a polymeric matrix filled or coated with bioactive glasses, glass ceramics and calcium phosphates, that combine the advantages of the two types of materials. The polymers used as the matrix can be natural materials – polysaccharides, such as chitin and chitosan, and proteins, such as collagen – or synthetic polymers, such as saturated aliphatic polyesters – polylactic acid (PLA), polyglycolic acid (PGA), polycaprolactone (PCL) and polyhydroxyalkanoates [1]. PLLA is a biodegradable synthetic polymer approved by the US Food and Drug Administration for clinical use (e.g. biodegradable sutures) and well known for its biocompatibility and adjustable physical and mechanical properties [1].

In terms of filler, bioactive glasses such as Bioglass® have been used as an inorganic filler in different structures for bone replacement to promote bioactivity, due to their ability to bond bone in vivo through the development of a bone-like apatite layer on the surface of the glass [3–8]. Additionally, they have been found to enhance the mechanical properties of scaffolds [3,6,7], support osteoblast growth and differentiation [6], induce osteogenic differentiation of marrow stromal stem cells [9], activate some osteoblast genes involved in bone growth [10] and stabilize the pH of the surrounding environment of the polymer to counteract the acidity of crystalline degradation products [2].

The processing technique is one determinant in obtaining a morphology that satisfies the above mentioned requirements of porous scaffolds and several techniques have been developed, including solvent casting [11], thermally induced phase separation (TIPS) [12–19], microsphere sintering [20], solid free-form and other porogen methods [1].

TIPS followed by freeze-drying has been used in the production of scaffolds, allowing the fabrication of highly porous structures (>95%) with pore morphologies that can be tailored by varying the preparation conditions [12–18]. In a typical procedure for

* Corresponding author. Tel.: +351 234 370 240; fax: +351 234 425 300.
E-mail address: helena.fernandes@ua.pt (M.H.V. Fernandes).

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scaffold production a polymer solution is prepared by dissolving the polymer in a solvent system at a determined temperature. The solvent system may comprise a polymer miscible solvent or a mixture of a miscible solvent with an immiscible solvent (i.e. non-solvent) to favour phase separation. The polymer solution is then poured into a mould and quenched at a lower temperature than the temperature used for dissolution of the polymer. The system is subsequently frozen and the solvent removed by freeze-drying. The resultant morphology of the scaffold depends on the final thermodynamic state of the polymer solution and on the rate of heat transfer during phase separation. Fig. 1 shows a schematic phase diagram for a general binary polymer/solvent system. The upper solid curve is to the binodal curve, the dashed curve the spinodal one. Depending on the quenching end point, liquid–liquid demixing proceeds according to different mechanisms. At temperatures between the binodal and spinodal curves the system is metastable with respect to infinitesimal composition fluctuations and phase separation occurs by a nucleation and growth mechanism. This mechanism induces the formation of a poorly interconnected structure with bead-like pores. On the other hand, for temperatures below the spinodal curve the system is unstable against infinitesimal fluctuations in composition and, consequently, the solution separates spontaneously in two phases, following a spinodal decomposition mechanism. Basically, it starts with small composition fluctuations and proceeds with a decrease in the Gibbs energy of the system, as one part of the system becomes more concentrated at the expense of another. At the end a well-interconnected open structure is obtained, which is desirable for scaffold preparation. Further details about spinodal decomposition can be found in van de Witte et al. [21].

Despite the advantages of PLLA and bioactive glasses, combination of the two materials for the preparation of scaffolds by TIPS for tissue engineering purposes has seldom been reported in the literature [3,11,12]. The main results reported have referred to the preparation of PLLA/bioactive glass scaffolds exhibiting a pore size of ~100 μm and their ability to exhibit in vitro bioactivity and in vivo biocompatibility with bone cells [3,11,12]. However, to the best of the authors knowledge the effect of bioactive glasses on the morphology of the resulting scaffolds has not been addressed.

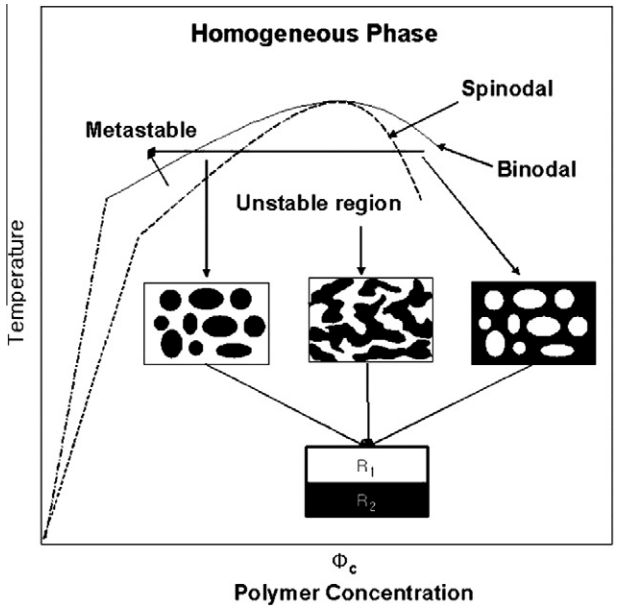

Fig. 1. Schematic temperature–composition phase diagram of polymer solution. R1, polymer-lean phase; R2, polymer-rich phase.

In the present study an alternative method for the preparation of macroporous composite scaffolds of poly(L-lactic) acid (PLLA) and bioactive glass (BG) for bone regeneration based on the TIPS methodology is proposed, without the use of possibly toxic surfactants. The effect of quenching time and BG content on the development of a tailored porous structure during phase separation has been investigated. The influence of these parameters on crystallization of the polymer has been studied, since it may restrict phase separation development in the scaffold. The potential bone bonding ability of the scaffolds was evaluated by in vitro acellular immersion tests in simulated body fluid (SBF).

2. Materials and methods

Poly(L-lactic) acid (PLLA Purasorb® PL39 with an inherent viscosity of ~3.9 dl g^{-1}) was obtained from Purac Biochem, The Netherlands, and used without further purification. 1,4-Dioxane (Panreac Quimica SA) was used as received. Distilled water purified using Millipore® Milli Q equipment was employed.

2.1. Preparation of bioactive glass particles

A BG from the $3CaO{\cdot}P_2O_5$–MgO–SiO_2 system with the composition 33.2 wt.% CaO, 28.2 wt.% P_2O_5, 15.6 wt.% MgO and 23.0 wt.% SiO_2 was fabricated by the traditional melt quenching method. The glass was prepared from a batch mixture of reagent grade $Ca(H_2PO_4)_2$ (Fluka, ⩾85% pure), $CaCO_3$ (Fluka, ⩾99% pure), MgO (Fluka, ⩾97% pure) and SiO_2 (BDH). The raw materials were ball mixed in ethanol for 45 min and dried at 70 °C for 24 h. Batches of 80 g were melted in a platinum crucible in air at 1500 °C for 2 h and then quenched in water in order to produce a glass frit. The amorphous state of the obtained frit was confirmed by X-ray diffraction (XRD) using CuKα radiation (Rigaku Geigerflex Dmax-C). The BG frit was then ball milled (Fritsch Pulverizette) to achieve an average particle size of 10 μm (measured with a Coulter LS Particle Size Analyzer) and a BET surface area of 0.7 $m^2\,g^{-1}$ (measured by the nitrogen adsorption method performed with a Gemini Micromeritics instrument).

2.2. Preparation of PLLA/bioactive glass scaffolds

Porous PLLA–BG composites were then prepared by the TIPS technique, varying the bioactive glass content (10, 30 and 50 wt.%) and the quenching time (30, 60, 120 and 180 min). The preparation procedure consisted of dispersion of the bioactive glass under stirring in a 5.5% (w/v) PLLA/1,4-dioxane/water solution at 80 °C. As PLLA is insoluble in water, the polymer was first dissolved in 1,4-dioxane at 80 °C for ~1 h, until complete dissolution. Afterwards, bioactive glass powder and purified water were added (dioxane/water = 87/13) and stirred for 1 h until a homogeneous clear solution was obtained. Subsequently, the solution was quenched at 37 °C for different quenching times to allow phase separation.

The samples were then frozen at −17 °C in a freezer for 8 h and lyophilized (freeze-dryer model Lyph Lock 4.5, Labconco) at the freeze-drying temperature −50 °C and under a vacuum of 15×10^{-3} mbar for 3 days, to remove solvents and obtain macroporous scaffolds.

2.3. Characterization of the scaffolds

The morphology of the scaffolds was examined by scanning electron microscopy (SEM) (Hitachi S-4100, Japan) at an accelerating voltage of 25 keV, using gold-sputtered samples. The average

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