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A facile preparation of highly interconnected macroporous PLGA scaffolds by liquid–liquid phase separation II

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Abstract

A regular and well-interconnected macroporous (from 50 to 200 μm) poly(D,L-lactic acid-*co*-glycolic acid) (PLGA) scaffold was fabricated by means of the thermally induced phase separation (TIPS) method. Poly(L-lactic acid) (PLLA) was blended with PLGA to increase the viscosity of polymer solution; a block copolymer of poly(ethylene glycol) (PEG) with PLGA was added as a surfactant to decrease the interfacial tension between the polymer-rich and polymer-lean phases. The effect of TIPS parameters including the concentration of diblock copolymer and PLGA/PLLA ratio was also studied. The cloud-point curve shifted to higher temperatures with both increasing the PLLA composition in the PLGA/PLLA blend and the PEG contents in the additives (PEG itself and PEG–PLGA diblocks). This shifting to higher temperature increases the quenching depth during phase separation. Addition of a PEG–PLGA diblock copolymer (0.5 wt% in solution) to the PLGA/PLLA (1/1) blend polymer in a dioxane/water solution stabilized the morphology development during TIPS with respect to interconnection and macropores, and avoided segregation or sedimentation in the late stage.

Keywords: Macroporous PLGA scaffold; Thermally induced phase separation (TIPS); Liquid–liquid phase separation

1. Introduction

After the loss or failure of bodily tissues or organs, traditional surgical treatment, such as implantation of a healthy organ from a donor, is limited by the problems of immune rejection from the patient and the number of available donors [1]. The use of cell transplantation ('tissue engineering') is under investigation as a strategy for tissue repair and organ replacement [2–6]. Transplanted cells, cultured from a patient's healthy tissues, can be implanted back without antagonizing the immunoisolation system. In culturing the cells, the shape of the scaffold, a temporary substrate to allow growth and specialization of the cell culture, plays an important role [7–10]. Biodegradable and biocompatible synthetic polymers, such as poly(lactic acid) (PLA), poly(glycolic acid) (PGA), and poly(D,L-lactic acid-*co*-glycolic acid) (PLGA), have been widely utilized as three-dimensional scaffolds [11–13]. Polymeric scaffolds must be porous enough to allow a high density of cells to be seeded, yet also possess sufficient mechanical stability and a well-defined network of interconnected pores to permit ingrowth into the implanted structure [9,14]. The optimum pore size of the scaffold required differs depending on the cells or tissues; for example, pore sizes close to 20 μm are required for the ingrowth of fibroblasts and hepatocytes [15], from 50 to 150 μm for skin regeneration [16], and in the range of 100–150 μm for bone regeneration [17,18].

Numerous techniques have been developed for fabricating polyester scaffolds, including porogen leaching/salt leaching, emulsion freeze-drying, gas expansion, fiber bonding, and phase separation [19–23]. Recently, the method of freeze-drying through thermally induced (liquid–liquid) phase separation (TIPS) was developed for the preparation of biodegradable polyester scaffolds [21,23–28]. TIPS and freeze-drying were used to prepare a three-dimensional macroporous poly(L-lactic acid) (PLLA)

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scaffold [23–30]. The TIPS technique provides a scaffold with a uniform pore size and high degree of interconnection, various morphologies, and good mechanical properties. The morphology can be controlled by several experimental parameters such as the quenching temperature, quenching rate, quenching period or aging time, polymer concentration, solvent to non-solvent ratio, molecular structure, and added surfactant or porogens [29–33].

Our group has reported the fabricating techniques based on liquid–liquid TIPS of polyester ternary systems to prepare macroporous PLA or PLGA scaffolds with highly interconnected structures and pore sizes ranging from 50 to 300 μm, suitable for certain cell cultures. Previously we prepared highly interconnected macroporous PLLA scaffolds with pore sizes of 50–150 μm by controlling the crystallization of PLLA during the coarsening process [29]. In other work, we found that the pore size of PLLA scaffolds with interconnected structures could be increased to greater than 200 μm through adding certain ionic compounds, surfactants, and triblock Pluronics [30,31,34].

In a preceding paper, we reported on the fabrication of a PLGA scaffold with pore sizes greater than 50 μm from PLGA/dioxane/water ternary systems. However, larger pore sizes and good interconnections between the pores were hard to get and the morphology development during quenching process was not stable because of the low viscosity of the PLGA solution [32].

In this study, the effect of blend with PLLA and the adding a PEG–PLGA diblock copolymer was investigated in order to get regular and highly interconnected macroporous PLGA/PLLA scaffolds with pore sizes greater than 200 μm. The scaffold morphology was also investigated by adjusting some of TIPS parameters.

2. Experimental

2.1. Materials

PLGA (High IV 50/50 lactic acid/glycolic acid (LA/GA); number-average molecular weight M_n 1.3×10^5; inherent viscosity $\sim$0.73 dL g^{-1}) was purchased from Alkermes. PLLA (Lacty 5000; M_n 2.18×10^5; PDI 1.55) was purchased from Shimadzu. 1,4-Dioxane and deionized water were a good solvent and non-solvent for PLGA/PLLA. Monomethoxy poly(ethylene oxide) (PEO; M_n 5000, PDI 1.1) was purchased from Aldrich and purified before use by dissolution in dry chloroform then precipitation from *n*-hexane. L-Lactide (Boehringer Ingelheim) was purified before use by recrystallization from thoroughly dried ethyl acetate under a dry nitrogen atmosphere and sublimation. PEG–PLGA diblocks (Table 1) were synthesized and characterized as reported previously [35].

Table 1
Characterization of the synthesized diblock copolymers

	Copolymer	Block M_n/g mol^{-1} [a]
Diblock1	PEG–PLGA	5000–2848
Diblock2	PEG–PLGA	5000–4532

[a] Number-average molecular weight calculated from ^{1}H NMR measurements.

2.2. Phase diagram

The cloud-point curves of the PLGA/PLLA blend in the presence of PEG or PEG–PLGA diblocks were determined by visual turbidimetry. PLGA/PLLA (3, 6, 9, and 12 wt%) and PEG or PEG–PLGA diblocks (0.2, 0.5, or 1 wt% in whole solution) were added to a 4 mL vial tube, equipped with a magnetic stirrer and 1,4-dioxane/water mixture (87/13 wt/wt) as solvent, and then dissolved at 58 °C for 3 h. The homogenous PLGA/PLLA solution was reheated to about 10 °C above the expected cloud-point temperature, then slowly cooled in steps of 1 °C, allowing the system to equilibrate for 10 min at each new temperature. The cloud-point was reported at the temperature at which the clear solution became visually turbid. The gelation point was determined by inverting the vial horizontally after it had been maintained for 10 min at a constant temperature, as described previously [29].

2.3. Preparation of PLGA/PLLA scaffolds

PLGA/PLLA (9 wt%) solutions in 1,4-dioxane/water (87/13 wt/wt) containing PEG or PEG–PLGA diblocks (0.2, 0.5, or 1 wt%) were prepared. The sample was reheated to 15 °C above the measured cloud-point temperature, then placed into a water bath preheated to the quenching temperature. The sample remained for 2, 10, 30, 60, or 120 min at this temperature. The annealed sample was directly immersed in liquid nitrogen for 1 h, and then one small hole was cut in the cap of vial to allow the solvents to depart. The freeze-drying was performed at −77 °C and 7 mTorr for 3 days in order to remove solvents and thereby obtain the macroporous scaffolds.

2.4. Morphology characterization

The macroporous morphology of the scaffolds was observed using scanning electronic microscopy (SEM, Hitachi S-2400). Fracture-frozen cross-sections of the scaffold were mounted on an Al stub covered with a carbon adhesive and then coated with Pt particles.

The size of pores PLGA/PLLA scaffold and PLGA scaffold (of the previous paper) were measured from micrographs to compare the size development during the fabrication. The average value from at least five pores was calculated from magnified micrographs.