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Fabrication of polycaprolactone nanofibrous scaffolds by facile phase separation approach

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ABSTRACT

Three-dimensional polycaprolactone (PCL) scaffolds with spherulite and nanofibrous structures were fabricated for the first time by thermally induced phase separation from a ternary PCL/dioxane/water system. Moreover, the effects of polymer concentration, aging temperature and the ratio of dioxane to water on the morphology of nanofibrous scaffolds were investigated. The result revealed that gelation, aging temperature, and ratio of solvents significantly influenced the formation of the unique spherulite and nanofibrous structures. The apatite-formation ability test showed relatively rapid growth of carbonate hydroxyapatite in the nanofibrous PCL scaffold with macropore compared to the other two scaffolds with smooth structure and nanofibrous structure without macropore, respectively, indicating good apatite-formation ability of the macroporous and nanofibrous PCL scaffolds.

1. Introduction

Tissue engineering has attracted remarkable attention because it exhibits an excellent potential for the regeneration of damaged tissue, circumventing the limitations of autologous tissue repair. Tissue engineering strategy requires the use of biodegradable scaffolds providing structural support and acting as reservoir for bioactive molecules. Recently, nanofibrous scaffolds have attracted significant interest in the field of tissue engineering [1,2], mainly due to their imitation of extracellular matrix (ECM) collagen. Collagen fibers, the major ECM component in tissues, have diameters ranging from 50 to 500 nm. According to the literature, the synthetic biodegradable nanofibrous scaffolds act as promising candidates for improving cell adhesion, proliferation, migration, and differentiation in various cell types [3]. Moreover, an increase in protein adsorption in the nanofibrous scaffolds leads to an increase in cell attachment, essential for proliferation, migration, and differentiation [4]. Among the synthetic biodegradable polymers, polycaprolactone (PCL) is biodegradable aliphatic polyester which has been approved by the Food and Drug Administration for certain human clinical applications, such as surgical sutures and some implantable devices [5]. Moreover, it is an ideal scaffold material because of its biocompatibility, nontoxicity for organism, gradual resorption after implantation, and good mechanical properties. PCL-based scaffolds act as promising candidates for tissue engineering to carry cells or growth factors and act as templates for tissue regeneration [6–9].

Many literatures demonstrated that the micro-nano or nano structure surface of the scaffold enhanced the adhesion, proliferation, and phenotype of cell better than the scaffold with smooth surface [10–12]. Moreover, nanofibrous scaffold affects phenotype of many types of cells such as nerve cell, hepatocyte, and fibroblast [13,14]. Ruckh et al. demonstrated the fabrication of nanofibrous PCL scaffolds by electrospinning technique [15]. The results indicated that 3D synthetic biodegradable nanofibrous scaffolds acted as excellent framework for enhanced cell adhesion, viability, and increased levels of alkaline phosphatase activity compared to the smooth PCL substrates. The investigations revealed that calcium phosphate mineralization was substantially accelerated on nanofibrous scaffolds compared to the smooth PCL.

Till date, three basic techniques have been used to fabricate scaffolds with nanofibrous structure, namely electrospinning, self-assembly, and thermally induced phase separation (TIPS) [16,17]. The electrospinning techniques produce polymer fibers with diameters ranging from nanometers to micrometers scale; however, the process involves a significant challenge in creating three-dimensional (3D) scaffolds with well-defined pore architecture and complex geometries [2,18]. Although, molecular self-assembly is a fairly new technique for designing nanoscale scaffolds, it demonstrates limited ability to control the pore size and structure, essential for cell incorporation, migration, and proliferation. Moreover, an improvement in the mechanical strength of self-assembled scaffolds is required before employing them for tissue engineering applications [2]. TIPS has been effectively used to fabricate nanofibrous scaffolds

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because it involves simple equipment, easy operation, and controllable 3D pore arrangement similar to natural ECM structure.

In this study, 3D PCL scaffold with spherulite and nanofibrous structure was fabricated by TIPS from a ternary PCL/dioxane/water system. The effects of gelation process, gelation temperature (T_{gel}), and ratio of dioxane to water on the morphology of designed nanofibrous scaffolds were investigated, and the structure–activity relationship of nanofibrous PCL scaffold was examined.

2. Materials and methods

2.1. Materials

PCL with an inherent viscosity of 1.68 dL g^{-1} was purchased from Jiangsu Youli Technologies Ltd. All other reagents and solvents with analytical grade were purchased from Sinopharm Chemical Reagent Co., Ltd.

2.2. Fabrication of nanofibrous PCL and macroporous and nanofibrous PCL scaffolds

The nanofibrous PCL scaffolds were fabricated by liquid–liquid phase separation from a PCL/dioxane/water ternary system. A given mass of PCL (5%, 10%, and 15%) was dissolved in dioxane/water mixed solvents with different mass ratios (90/10, 88/12, and 85/15), respectively, at 40 °C. By cooling the transparent polymer solution in a rate of 2 °C/30 min, the cloud point was determined as the temperature when the solution became turbid. The gelation temperature was determined as the solution could no longer flow when the sample was held at a temperature.

5 ml of PCL transparent solution was poured into a beaker mold (3.5 cm in diameter and 5 cm in height). Subsequently, it was quenched to a designed temperature (−8, −4, 0, 4, 8, and 12 °C) and aged for 2 h, followed by quenching to −40 °C for another 2 h to completely freeze the samples. During this period, the solution would come in the form of solid state. The scaffold (3.5 cm in diameter and 1 cm in thickness) was obtained after lyophilization under 0.940 mbar at −54 °C for three days (FD-1A-50 freeze dryer, Beijing Boyikang Experimental Instrument Co., Ltd.). To study the effect of aging on the morphology of the sample, in the gel status, 10% (w/w) PCL/dioxane/water solution was directly quenched to −40 °C and maintained for 2 h followed by lyophilization under 0.940 mbar at −54 °C for three days.

Moreover, macroporous nanofibrous PCL scaffolds were also fabricated for comparison, by a technique combining the unique phase-separation method with sugar leaching process, from PCL/dioxane/water ternary system. Mixture solution (50%) was obtained by dissolving a certain amount of sugar (diameter in 150–500 μm) in clear solution of PCL (10%, w/w) at room temperature with stirring. The resulting mixture solution was quenched to −4 °C and maintained for 2 h, followed by quenching to −40 °C for another 2 h to completely freeze the samples. Subsequently, the samples were immersed in cold ultrapure water (4 °C) for three days for leaching out the sugar by changing the water three times per day. The sample so obtained was freeze-dried under 0.940 mbar at −54 °C for three days.

2.3. Characterization of nanofibrous PCL scaffolds

The morphology of PCL scaffolds prepared under different conditions was observed by scanning electron microscopy (SEM) (FE-SEM, JSM-7500 F, JEOL Ltd.) at 5 KV. The samples were fractured after liquid nitrogen treatment and coated with gold for 150 s using a sputter coater (AUTO FINE COATER, JFC-1600, JEOL Ltd.).

The precipitated apatite layer of nanofibrous PCL scaffolds after immersion in the SBF were analyzed using a Philips X'pert MPD X diffractometer using Cu Ka generated at 40 KV and 40 mA. The samples were scanned from 10° to 90° with a step size of 0.02° and a count rate of 3.0°/min.

FTIR spectra were measured to confirm the formation of nanofibrous PCL scaffolds after immersion in the SBF. A nicolet Avatar 360 spectrometer, with KBr pastille, was used for FTIR characterization. The analysis range was from wave number 4000 to 400 cm^{-1}.

2.4. Porosity test

The porosity of the scaffolds was determined by a simple method [19]. To determine the porosity, different samples were dried at 40 °C for a day. After weighing (W_s), each sample was placed into a pycnometer filled with ethanol, with the weight of pycnometer and ethanol taken together as W_1. Subsequently, the pycnometer was placed into a vacuum container to extract the air out of the sample, thus pushing ethanol into the space originally occupied by air bubble. During the vacuum process, the fluid level in the pycnometer fell down. Therefore, the pycnometer was taken out and filled up, and the entire weight (W_2) was taken. Subsequently, the sample was taken out and the surface ethanol was dripped back into the pycnometer to obtain the weight of the remaining ethanol and the pycnometer (W_3). The porosity can be calculated from the following equations [20], where ρ is the density of ethanol:

$$\text{Scaffold volume}: V_s = (W_1 - W_2 + W_s)/\rho$$

$$\text{Pore volume}: V_p = (W_2 - W_3 - W_s)/\rho$$

$$\text{Porosity}: \varepsilon = V_p/\left(V_p + V_s\right) = (W_2 - W_3 - W_s)/(W_1 - W_3)$$

2.5. Apatite-formation ability

Bioactivity is a critical factor in facilitating the chemical fixation of biomaterials to bone tissue, and ultimately the in vivo success of the bone grafting material [21,22]. The nanofibrous PCL scaffold and macroporous and nanofibrous scaffold were immersed in a 1.5 simulated body fluid (1.5SBF) with ionic concentration nearly equal to human blood plasma. 1.5SBF was prepared as described in the literature [23], the pH of the 1.5SBF is 7.4 and ionic concentrations of 1.5SBF and human plasma was listed at Table 1. The scaffolds were immersed in SBF for 14 days. Finally, the samples were rinsed thoroughly with distilled water and dried at 40 °C.

3. Results

3.1. The cloud point and gelation point of the PCL

Fig. 1 shows the dependence of the cloud points and the gelation points on the polymer concentrations and the solvent composition with different mass ratios. The cloud points and gelation points increased as the increase of the polymer concentration and the water content in mixed solvents. The dependence on water content was greater than those of the polymer concentration.

Table 1
Ion concentrations of SBF and human plasma (mmoL/L).

	Na^+	K^+	Mg^{2+}	Ca^{2+}	Cl^-	HCO_3^{2-}	HPO_4^{2-}	SO_4^{2-}
Human plasma	142.0	5.0	1.5	2.5	103.0	27.0	1.0	0.5
1.5SBF	213.00	7.50	3.80	2.30	6.30	223.00	1.50	0.75