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Polycaprolactone scaffolds or anisotropic particles: The initial solution temperature dependence in a gelatin particle-leaching method

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ABSTRACT

A porogen-leaching method was applied to intend fabrication of polycaprolactone (PCL) scaffolds. Following with a routine solution infiltration, freeze-drying and porogen-leaching process, the porous scaffolds were normally prepared at an initial solution temperature of 25 °C. However, the PCL anisotropic particles with the smooth and fuzzy surfaces toward the gelatin porogen and the solution, respectively, were unexpectedly obtained when the initial solution temperature was maintained at 37 °C. The freezing temperature was a governing factor for formation of the different PCL products too, while the coarsening time and the PCL concentration within 10–20% had no substantial influence. The PCL anisotropic particles are highly crystallized than the PCL raw materials. To clarify the intrinsic mechanisms, the temperature, cloud point, crystalline ability, and particle size in the solution were quantified. It is demonstrated that the sponges are formed by the traditional liquid–liquid demixing for the 25 °C solution, whereas the anisotropic particles are obtained by the solid–liquid demixing for the 37 °C solution and under the assistance of gelatin particles as nucleation sites.

1. Introduction

As a crucial factor in tissue engineering and regenerative medicine, the scaffold functions as a physical template to support cell adhesion, proliferation and differentiation, and provide channels for the exchange of nutrients and wastes [1–8]. The materials used for fabricating the 3-D scaffolds can be both nature-originated and synthetic. Polycaprolactone (PCL) is a kind of semi-crystalline and biodegradable polymers obtained through petrochemical process, and has been widely used for bone and cartilage tissue engineering [9–13], vascular reconstruction [14,15], and bio-artificial liver and so on [16,17]. Its crystallization property enriches its versatility in making many functional materials and devices [18–21].

Recently, many methods such as solvent-casting/particle-leaching [22,23], thermally induced phase separation (TIPS)/freeze-drying [24–27], electrospinning [28,29], and supercritical fluid-gassing [30,31] have been applied for preparation of the 3-D scaffolds. Among which the particle-leaching method is frequently adopted because of the controlled pore size and distribution, which is mainly determined by the size of the porogens, and the controlled morphology on the pore walls, which is a key factor to determine the cell and tissue response *in vitro* and *in vivo* [2,32,33]. So far many kinds of particles such as NaCl [34], sugar [35], gelatin [24], and paraffin [36] have been used in the preparation of porous scaffolds. For example, Wan's group used the NaCl particles to fabricate a chitosan-g-PCL scaffold with a gradient pore size. The pore size and porosity increase gradually along with the longitudinal direction and can be controlled by selecting particles with different sizes [37]. Chen's group used ice particulate templates to prepare funnel-like collagen sponges for cartilage tissue engineering [38]. Our group used gelatin particles to fabricate poly(L-lactic acid) scaffold [39] poly(lactide-co-glycolide) sponge for cartilage tissue engineering [40]. As demonstrated previously, introduction of gelatin can simultaneously improve the positive interaction between the materials and cells without any potential side effects. Moreover, the annealing at high temperature and humidity is optionally used to bind the gelatin particles, so that the replica pores can have good interconnectivity.

When the scaffold is fabricated by the particle-leaching method, TIPS process is generally accompanied, resulting in tiny pores on the walls of macro-pores and thereby a hierarchical structure of the scaffold. The TIPS process is mainly determined by four aspects, i.e. polymer, solvent with high boiling point and low molecular weight, cooling process and diluent-removing process [41]. Many factors such as material composition, solvent composition, polymer

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concentration, frozen temperature, quenching route and coarsening time are used to adjust the TIPS process to obtain the scaffolds with different microstructures [38,42–45]. Therefore, the combination effects of the porogen-leaching and the TIPS will decide the scaffold microstructures. For example, Ma's group reported the fabrication of scaffolds with macro-pores by using sugar particles as porogen, and the pore walls of the scaffold can be adjusted from nanofibers, partial nanofibers to smooth surface [35,46]. It is widely proved that the nanoscale structure of the scaffold has a great impact on the cell responses such as adhesion [3,47], proliferation [48], differentiation [49–51] and gene expression [52]. Therefore, it is very important to fabricate the scaffold with different topographical features by adjusting the fabrication parameters.

In this study, it is found for the first time that the anisotropic PCL particles or sponges are formed during the scaffold fabrication by the method of gelatin particle-leaching. The microstructure is mainly governed by the initial temperature of the PCL/tetrahydrofuran solution, which is a factor often neglected in traditional TIPS. Attention is then paid to the influencing factors and the intrinsic mechanisms, which are finally clarified based on the experimental results and theoretical analysis. This finding is not only important for developing a scaffold of new microstructures, but also attractive for basic science and materials chemistry since the anisotropic particles have very unique properties yet are difficult to fabricate in big batch [53].

2. Experimental section

2.1. Materials

Polycaprolactone (PCL, M_n = 80 kDa) was purchased from Sigma–Aldrich. Gelatin was obtained from Chinese Medicine Company and sieved to collect the particles with a size of 280–450 μm before use. Camphene (with a purity of 97%) was purchased from Aladding Company Ltd., China. All other reagents were of analytical grade and used as received.

2.2. Preparation of PCL scaffolds

The gelatin template was fabricated via a method reported previously [54]. Briefly, the gelatin particles within a size of 280–450 μm were added into a cylindrical glass vial with a diameter of 12 mm, followed by a slight press on the top, and then treated with saturated water vapor at 70 °C for 4 h. After freeze-dried, the gelatin template was immersed into PCL/tetrahydrofuran (THF) solution and maintained under a low pressure of 0.07–0.08 MPa to evolve the trapped air bubbles. When the pressure was released, the polymer solution was spontaneously infiltrated into the cavities between the gelatin particles. The PCL/THF solution-filled gelatin template was kept at a certain temperature (noted as the initial temperature) for 3 h, coarsened in a −20 °C refrigerator for a certain period of time, and then freeze-dried. The gelatin template was leached by incubation in 300 ml Millipore water at 37 °C for 3 d. Finally, the PCL scaffold was obtained after freeze-drying.

2.3. Morphological characterization

The morphology of the PCL porous scaffold or PCL particles was characterized by scanning electron microscopy (SEM, SIRION-100, the Netherlands). To view the cross-sectional morphology, the PCL scaffold was frozen in liquid nitrogen for 5 min and then cut by a razor blade before gold-coating. The PCL particles were also observed under a polarized optical microscope (Olympus BX51, Japan).

2.4. X-ray diffraction

The PCL particles were characterized by X-ray diffraction (X'Pert PRO, the Netherlands) at a glancing angle from 3° to 60°, and with the supplied voltage and current of 40 kV and 40 mA, respectively. Samples were exposed at a scan rate of 0.0167°/10 s.

2.5. Differential scanning calorimetry

The differential scanning calorimetry (DSC) analysis was carried out using a Perkin–Elmer Pyris 1 instrument. The PCL/THF solutions were heated to 70 °C to eliminate the thermal history, and then decreased to −60 °C at a rate of −10 °C/min.

2.6. Dynamic light scattering

The variance of mean diameter of 10% PCL/THF solution was monitored by using a Brookhaven BI-200SM dynamic light scattering instrument. The PCL/THF solution was heated to 70 °C and then decreased to −5 °C by an ethanol circulating cooling system. Data were collected at the predetermined temperature.

3. Results and discussion

The porogen-leaching method has been very frequently employed to prepare the spongy scaffolds with good control over the macroscopic shape and microstructure as well as other physicochemical properties [35,39,46]. Particularly, the gelatin particles/spheres are demonstrated as one of the most promising porogens to prepare the porous scaffolds with very good interconnectivity between pores and cytoviability due to the simultaneous entrapment of gelatin molecules on the pore walls [40,54]. Therefore, in this study the gelatin particles template was infiltrated with the PCL/THF solution of different concentrations and initial solution temperatures to prepare the PCL scaffold after freeze-drying and particle-leaching in water.

3.1. Influence of initial temperature and coarsening time

It is known that the freeze-drying can simultaneously induce the so-called phase separation, resulting in smaller pores on the walls of big pores which are the replica of the gelatin particles (280–450 μm) [55]. These small pores can improve the connectivity between the big pores and enhance the exchange of nutrients, metabolic products and oxygen etc., and thus are important for the overall performance of the scaffolds. Among the various factors influencing the formation and size of the smaller pores during freeze-drying, the initial temperature and polymer concentration take the major roles since they can change the quenching rate and the location in the binodal/spinodal phase separation curve, and thereby influence significantly the phase separation behaviors [56].

For this context, the systems of infiltrated PCL/THF solution and gelatin template were firstly maintained at 25 °C and 37 °C to reach the equilibrate state, and then moved to a refrigerator at −20 °C, allowing the coarsening of the solution for 3, 8 and 20 h, respectively (Fig. 1). When the initial solution temperature was 25 °C, PCL scaffolds with a porous structure were obtained regardless of the coarsening time (Fig. 1a–c). However, the size of the small pores was gradually increased along with the prolongation of the coarsening time, accompanying with the thickening of the walls of big pores. It was unexpected, however, that no scaffold was obtained when the initial solution temperature was maintained at 37 °C. Instead, only particles were formed regardless of the coarsening time (Fig. 1d–f). The average size of the PCL particles increased