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Fabrication of chitosan-*g*-polycaprolactone copolymer scaffolds with gradient porous microstructures

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Abstract

Chitosan-*g*-polycaprolactone copolymer (CPC) with a relatively low degree of substitution of polycaprolactone (PCL) and a PCL content of around 50 wt.% was first synthesized. CPC was further used to fabricate porous scaffolds with a novel processing method. Basing on a layer-by-layer assembly technique and choosing salt as porogen, these produced scaffolds showed interconnected porous microarchitectures with gradually increasing pore size and porosity along the longitudinal direction. By selecting an appropriate solvent and optimizing processing conditions, the resulting scaffolds would have various pore sizes and porosities which changed from ~85 to ~390 μm and from ~66% to ~91%, respectively.

Keywords: Chitosan-*g*-polycaprolactone copolymer; Porous scaffold; Gradient microstructure

1. Introduction

Three-dimensional (3D) porous polymer scaffolds have been widely used for tissue engineering of biological substitutes, where they provide the biomechanical support for the seeded cells until they are well organized into a functioning tissue [1]. It has been stated that in addition to matrix chemistry, the microstructures of scaffolds could exert a decisive effect on the developing tissue in culture [2,3]. Many investigators have reported optimum pore size ranges for the different kinds of cells or tissues [4]. In articular cartilage regeneration, porous scaffolds with various pore sizes and porosities at different layers are frequently required, and various biomechanical properties for final engineered tissue could be achieved by using this type of scaffolds [5]. However, it is usually difficult to fabricate well-constructed scaffolds with gradient pore sizes and porosities using common processing techniques, such as particulate-leaching and freeze-drying methods, even though 3D printing [6] and spinning [7] techniques have also been recently tried.

In the present study, we have fabricated a type of chitosan-*g*-polycaprolactone copolymer scaffolds with gradient pore sizes and various porosities gradually increased along the longitudinal direction of the scaffold by using a newly developed method. This technique is consisted of a layer-by-layer assembly and a followed particulate-leaching process. It was found that the pore size ranges of the scaffold could be easily controlled and the resulting scaffolds could have a predesigned microstructure.

2. Experimental

2.1. Materials

Chitosan flakes were purchased from Fluka. To obtain highly deacetylated chitosan, the purchased chitosan flakes were treated for 1 h in a 50 wt.% NaOH solution at 100 °C, and the alkali treatment was repeated twice using a method described elsewhere [6]. Its degree of deacetylation and viscosity-average molecular weight was determined as 93.1 (±2.1) % and 7.9(±0.18)×10^5 according to the reported method [7]. Trifluoroacetic acid-*d* (99.5 at.% D) and deuterium oxide (99.9 at.% D) were provided by Fisher. Caprolactone and all other chemicals were obtained from Aldrich and used without further purification. Chitosan-*g*-polycaprolactone copolymer (CPC) was synthesized using reported methods [8,9].

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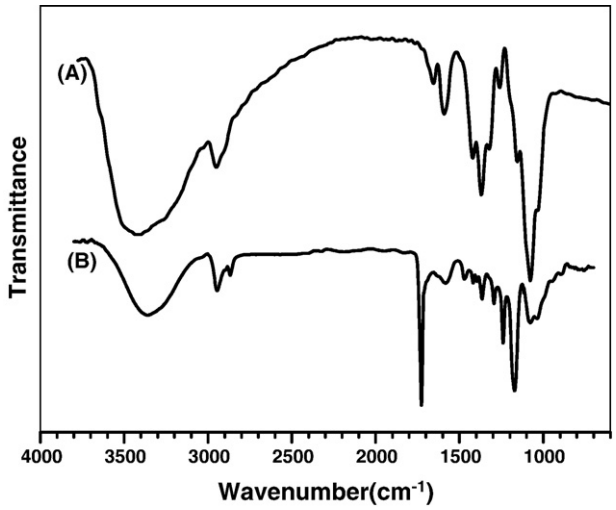

Fig. 1. FTIR spectra of (A) chitosan and (B) CPC.

2.2. Fabrication of scaffolds

Grained CPC powders were further cryogenically milled in a mill (SEPX 6750, METUCHEN) with liquid nitrogen, vacuum-dried and sieved into a range within 106–150 μm. Fine NaCl particles were prepared by milling them in a large analytical mill, and were then sieved into different groups with various mesh sizes from 30 to 80. CPC powders and salt particles were mixed together at predetermined weight ratios in a ball mill for 48 h using zirconia as milling media. A thin layer (around 1 mm in thickness) of mixed powders was first spread and pressed tightly on a circular stainless steel mould having a movable bottom, and then a motor-controlled syringe reciprocally moved above the powder bed and deposited very small solvent (dimethyl sulfoxide, DMSO) droplets in a way so that the powders were bound into many parallel filaments in one layer. After one layer was completed, the bottom of the mould was lowered (around 1 mm), turned a given angle (for example, 36° at each layer for a 5-layer scaffold) and a new layer of powders with an increasing weight ratio and particle size of salt was spread and pressed, followed by additional deposition of solvent droplets. The fabrication was repeated until the required constructs were built. The so obtained samples were placed into a sealed flake for at least 5 h and dried in vacuum at 40 °C for one day. These samples were leached in 1.0 wt.% NaOH solution to remove salt, repeatedly washed with distilled water until neutral pH was reached, lyophilized at −75 °C (EYELA FD-5N freezer) for 2 days and totally dried again in vacuum.

2.3. Characterization

The infrared spectra of CPC were recorded on a Nicolet 510P FTIR spectrometer with a resolution of 2 cm^{-1}, 32 scans, in transmission mode. All samples were prepared as KBr pellets and were scanned against a blank KBr pellet background. ^1H NMR measurements were performed on a Bruker Avance-500 (dmx-500) spectrometer at room temperature using a mixed solvent of D_2O and CF_3COOD (D_2O/CF_3COOD 90:10 v/v) following the reported method [10]. Weight percent of polycaprolactone (PCL) in CPC was measured via a gravimetric method described by Feng et al. [11]. Degree of substitution (DS) of PCL on the hydroxyl group of chitosan was determined employing a hydroxyl-titration technique [12].

Scaffolds were carefully sectioned into slides along the longitudinal direction, coated with gold–palladium and viewed using a Philips XL-30 SEM microscope. The average pore sizes of the scaffold section surfaces were measured from SEM images using an image analysis program (examining 100 different points in a 712×484 SEM image for each scaffold section). The porosity of the scaffold sections was measured using a technique described elsewhere [13].

3. Results and discussion

PCL has served for different medical applications for a long time. Like other biodegradable polyesters, PCL has a few major shortcomings such as hydrophobicity, lack of specific cell-recognition sites and acidic degradation products [14]. Several efforts [15,16] have been made to blend PCL with chitosan in order to comprehensively improve the properties of PCL because chitosan has functional groups on its backbone, shows high hydrophilic characteristic and is also a weak basic polyelectrolyte. But only limited miscibility was achieved since two main difficulties were encountered: (1) the melting processing

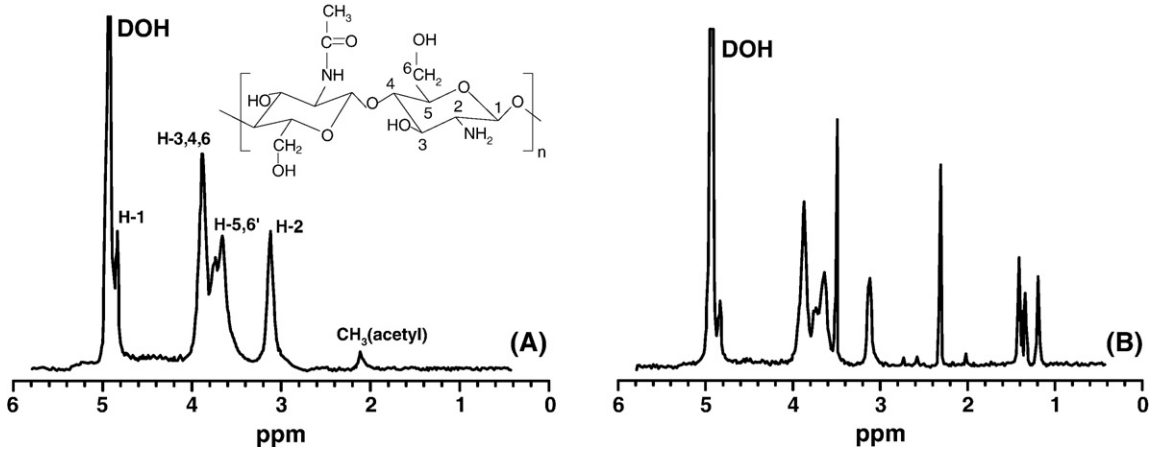

Fig. 2. ^1H NMR spectra for (A) chitosan and (B) CPC.