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ScienceDirect
Journal of Bionic Engineering 12 (2015) 565–574

Fabrication of Dual-pore Scaffolds Using a Combination of Wire-Networked Molding (WNM) and Non-solvent Induced Phase Separation (NIPS) Techniques

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Abstract

In this study, to fabricate dual-pore scaffolds with interconnected pores, Non-solvent Induced Phase Separation (NIPS) and Wire-Network Molding (WNM) techniques were combined. First, a mold with uniform slits was prepared, and needles were inserted into the mold. Subsequently, polycaprolactone (PCL) pellets were dissolved in tetrahydrofuran (THF) at a specified ratio. The slurry was mixed using hot plate stirrer at 1200 rpm for 24 hours at 40 ˚C. The PCL slurry was subsequently injected into the mold. Thereafter, to exchange the THF (solvent) with the ethanol (non-solvent), the mold was soaked in an ethanol bath. After removing the mold from the ethanol bath, the needles were removed from the mold. Consequently, dual-pore scaffold with interconnected pores was obtained. The surface morphology of the fabricated scaffolds were observed using Scanning Electron Microscope (SEM). Moreover, cell culture experiments were performed using the CCK-8 assay, and the characteristics of cells grown on the dual-pore scaffolds were assessed and were compared with the NIPS-based 3D plotting scaffold.

Keywords: tissue engineering, scaffold, polycaprolactone (PCL), Wire-Network Molding (WNM), Non-solvent Induced Phase Separation (NIPS)

doi: 10.1016/S1672-6529(14)60146-3

1 Introduction

Many studies have been performed to regenerate damaged tissue through the use of tissue engineering techniques. In tissue engineering, the scaffold is considered as an essential factor along with the cells and growth factors[1–4]. Moreover, for successful cell viability, 3D scaffolds should have necessary conditions, such as interconnectivity of pores, suitable mechanical strength, appropriate porosity, biocompatibility, and biodegradability[5,6]. A few representative synthetic polymers, which have been used in the field of tissue engineering, are PCL (polycaprolactone), PLLA (L-lactic acid), PLGA (L-lactide-*co*-glycolide), and PGA (polyglycolic acid)[7,8]. Among these polymers, PCL has a relatively high biocompatibility, biodegradability, and interesting mechanical strength characteristics[9,10]. Additionally, when using the synthetic polymers mentioned above, many fabrication methods have been proposed as follows: gas-foaming[11,12], freeze drying[13,14], salt-leaching[15–17], phase separation[18–22], 3D-plotting (printing)[23–25], stereo lithography[26], and laser sintering[27].

Scaffolds should have the appropriate three-dimensional (3D) architecture for the cell environment. Furthermore, dual-pore scaffolds with the interconnected pores (networks) are a promising scaffold to guide cell attachment and ingrowth[28]. In dual-pore scaffolds, global pores act as channels for the nutrient supply and waste discharge, and the local pores can provide the space for cell attachment and proliferation. Until now, the following dual-pore scaffold fabrication methods have been proposed: gas foaming and phase separation[29], a robocasting (robotic-dispense) technique and freeze-drying[30], bioactive sol-gel process[31], melt-spinning[32], Collagen/Hydroxyapatite composite

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technique[33], overrun and particle-leaching process[34], low-temperature deposition method[35], and a Non-solvent Induced Phase Separation (NIPS)-based technique[36]. Low-temperature deposition method is also excellent fabrication method. However, dioxane was used as solvent and 'support material' after deposition with utilization of refrigerator. Because the melting temperature of dioxane is about 10 Celsius degrees, dioxane is adequate to the process of Low-temperature Deposition Manufacturing (LDM). However, if the solvent is THF (Tetrahydrofuran), LDM is hard to be adopted because the melting temperature of THF is under −100 Celsius degrees. Therefore, in the viewpoint of solvent's diverse selection, LDM process has a certain limit. Among these methods, NIPS-based techniques could possibly be an excellent fabrication method. However, according to study[36] by Shin *et al*., the scaffolds could collapse due to the absence of support. Therefore, in this study, a NIPS-based technique was improved using a Wire-Network Molding (WNM)[17] technique to prevent the possibility of the scaffold collapsing.

First, using the WNM mold and PCL slurry dissolved in THF, modified NIPS-based dual-pore scaffolds were fabricated. Subsequently, the morphology of the fabricated scaffold was observed using Scanning Electron Microscope (SEM) to compare with an unmodified NIPS-based scaffold, which was used as a control scaffold and fabricated according to the process suggested by Shin *et al*.[36]. Additionally, through an *in vitro* experiment using Saos-2 cells, cell proliferation was analyzed using a CCK-8 assay.

2 Materials and methods

2.1 Materials

Polycaprolactone (M_n = 80,000, Sigma-Aldrich), ethanol alcohol absolute (99.9%, Daejung Reagents Chemicals), THF (99.5%, OCI Company Ltd.), stainless steel needles (Dongbang Acupuncture, Korea) and a stainless steel mold were purchased to fabricate the scaffold. Molds were designed using CATIA (V5R13) and fabricated using a wire-cutting process. Furthermore, Saos-2 cells were obtained from American Type Culture Collection for cell culture.

2.2 Fabrication of mold

To fabricate the dual-pore scaffolds using the WNM technique, a mold was designed using CATIA, which is a commercial Computer-Aided Design (CAD) program. Subsequently, using a wire-cutting process, a stainless steel mold was fabricated, as shown in Fig. 1. The thickness of sidewall part in the mold assembly was 2 mm, and the inner dimensions of the mold assembly were 25 × 25 × 16 mm^3. The bottom part of the mold assembly was 25 × 25 × 10 mm^3 and was designed to prevent leakage of the PCL slurry. For the sidewall part, 500 μm-wide slits were manufactured for needle insertion, as shown in Fig. 1. The pitch between slits was 1000 μm. Therefore, there was a 500 μm gap between the slits. First, the four sidewall parts having uniform slits were assembled to the mold assembly, as shown in Fig. 1. Then, the bottom part was assembled to prevent leakage of the PCL slurry. Through the slits, the prepared needles with 500 μm diameters were inserted to form a network shape, as shown in Fig. 1. The needles were stacked to form 8 layers, and the height of the needle network was 4 mm, which was the height of the fabricated scaffold. The top part (with dimension 25 × 25 × 25 mm^3) was assembled after injection of the PCL slurry during the fabrication process.

2.3 Scaffold characterization

To investigate the surface and cross-section morphology of fabricated scaffolds, SEM (JSM-5410, JEOL, Japan) was used. Fabricated scaffolds were coated with platinum for 60 seconds before morphology of scaffolds were analyzed. Coated scaffolds were placed on a stainless steel plate and observed in argon.

In cell-seeded scaffolds, porosity is an important parameter because the porosity indicates the total proportion of the space within the scaffold in which cell proliferation can take place. The porosity was calculated using Eq. (1) as:

$$\text{Porosity} = \frac{V_0 - (m/\rho)}{V_0} \times 100(\%). \quad (1)$$

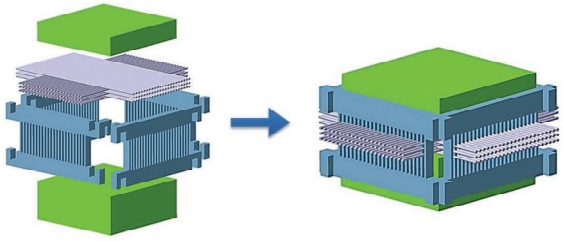

Fig. 1 A schematic of the mold set for the WNM-NIPS scaffold.