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Effects of heat-treatment on surface morphologies, mechanical properties of nanofibrous poly(propylene carbonate) biocomposites and its cell culture

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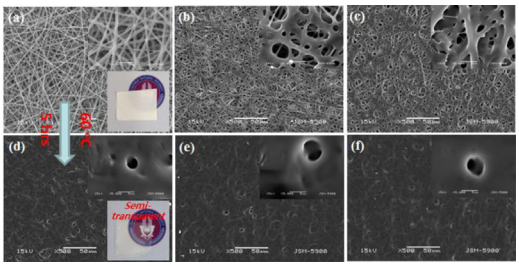
HIGHLIGHTS

- PPC nanofibers exhibited soft rubber-like features.
- Mechanics of PPC nanofibers were dramatically improved after heat-treatment.
- PPC/PLA composite nanofibers showed topographically peculiar morphologies.
- Biomedical applications as a scaffold tissue engineering.

GRAPHICAL ABSTRACT



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ABSTRACT

We report controlled surface morphologies, mechanical properties and cell culture of biomimetic pure poly(propylene carbonate) (PPC) and PPC/poly(lactic acid) (PLA) composite nanofibers prepared by sol–gel electrospinning. Pure PPC nanofibers exhibited soft rubber-like features with lower mechanical properties (elastic modulus ~59.7 MPa, tensile strength ~6.2 MPa), while the mechanical properties of as-spun PPC nanofibers were dramatically improved after heat-treatments at 60 °C. Furthermore, the PPC/PLA composite nanofibers exhibited topographically peculiar morphologies (in particular, *an embossing-like surface protrusions*), probably due to poor miscibility between PPC and PLA. CCK-8 assay results of cultured myoblasts clarified that pure PPC and PPC/PLA composite nanofibers were nontoxic to the cells, which therefore, suggest them to be used in biomedical applications as a scaffold for tissue engineering.

1. Introduction

The synthesis of poly(propylene carbonate) (PPC) was first reported by Inoue et al. [1]. The PPC, which has now been industrialized via the copolymerization of carbon dioxide and propylene oxide, has attracted much attention due to its good biocompatibility and biodegradability. The use of CO_2 as one of the monomers

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in the synthesis of PPC can not only overcome the shortage of petroleum resources but also contribute to reducing carbon dioxide pollution [2,3]. In general, PPC exhibits good tensile toughness and transparency. However, its inferior tensile strength and low glass transition temperature (T_g, 35–40 °C), due to the weak interchain interaction of this copolymer, are the major drawbacks to prevent it from widely practical application. Therefore, the reinforcement of PPC is in urgent demand to extend its applications [4,5]. For instance, melt blending of PPC with various biodegradable polymers, such as poly(3-hydroxybutyrate) (PHB) [6], poly(butylene succinate) (PBS) [7], poly(ethylene-co-vinyl alcohol) (EVOH) [8], and starch [9], has drawn much attention to obtain PPC materials with good biodegradability and improved mechanical properties. PPC could also be reinforced by inorganic or organic filler, such as $CaCO_3$ [10], montmorillonite [11], graphite oxide [12,13], glass fiber [14] and carbon nanotubes [15].

An ideal scaffold should possess a suitable surface chemistry that supports cell attachment, proliferation, migration and growth [16]. Additionally, it should serve as a biocompatible template for cell growth and aid in the differentiation of the cells, as well as supporting the production, organization and maintenance of an extracellular matrix [17,18]. In addition to being biocompatible, scaffolds are required to be composed of highly interconnected macro and micro-porous networks to facilitate cell migration and nutrient distribution. Recent papers indicated that cell migration was mainly controlled by the physical aspects although both physical structure (i.e., channels formed by aligned nanofibers or micro-patterned films) and chemical/biological agents (i.e., growth factors) facilitate cell adhesion and differentiation [19–21]. Nanofibers, mostly produced by electrospinning technique [22–24], show great potential for an optimal template for cell attachment, proliferation and growth, since they have small diameter, large surface-to-volume ratio, high porosity, and a similar morphology to native extracellular matrices. This method has been often utilized for producing scaffolds that mimic the morphological characteristics and biological function of the natural extracellular matrix, by providing an optimal template for cell attachment, proliferation and growth [25,26]. Several different polymers such as polyurethane (PU), poly(ε-caprolactone) (PCL), poly(lactic acid) (PLA), poly(glycolic acid) (PGA) and their copolymers have been successfully spun for musculoskeletal, never, skin, vascular and drug delivery applications [27–30]. In this work, we have studied the effects of heat-treatment on the surface morphologies and mechanical properties of pure PPC nanofibers depending on the time of heat-treatment. Further the biocompatibility and cell culture of the PPC/PLA composite nanofibers with unique topographical features was tested to investigate its potential application as a scaffold for tissue engineering.

2. Materials and methods

2.1. Materials

Poly(propylene carbonate) (PPC) of average molecular weight M_n ~100 kDa was kindly supplied by SK Innovation, Korea. Poly(lactic acid) 'Nature Works® PLA Polymer 3051D', average molecular weight 160 kDa, ratio 96% L-Lactide to 4% D-Lactide units was obtained from Nature works Minneapolis Minnesota (USA). Myoblast C2C12 cells (ATCC-CRL 1772) and Cell Counting Kit-8 (CCK-8), were purchased from American Type Culture Collection (ATCC) and from Sigma–Aldrich Chemical Co. (St. Louis, MO, USA), respectively. Dulbecco's modified Eagle medium (DMEM) from Gibco® life technologies, Grand Island, NY, USA and laboratory wares were purchased from Falcon Labware (Becton-Dickinson, Franklin Lakes, NJ, USA) respectively.

2.2. Fabrication of pure PPC and PPC/PLA composite nanofibers

The PPC polymers were dissolved in a mixture of chloroform and *N*,*N*-dimethylformamide (DMF) (3:7 by a weight ratio) as solvent, and then used for electrospinning. The optimum concentration was 35 wt%. The PPC/PLA composite nanofibers were prepared by sol-gel electrospinning method. For a successful sol-gel electrospinning, the pre-prepared PPC solution was mixed with PLA stock solution to produce the PPC/PLA mixtures with different contents of PLA ranging from 0.1 to 2.0 wt% (based on PPC mass), and then further stirred overnight. When increasing the PLA concentration above 2.0 wt%, we failed to electro-spin the PPC/PLA mixtures because of sever phase separation and/or gelation of PPC/PLA mixtures. Each mixture was supplied through a 10 ml glass syringe attached to a metallic tip with an inner diameter of 0.6 mm, and then electrospun onto a rotating metallic collector. To produce electrospun nanofibers, a high voltage power supply (CPS-60 K002V1, Chunpa EMT Co.) capable of generating voltage up to 80 kV was used as a source of electric field. Heat-treatment was performed at 60 °C for various times (1–10 h).

2.3. Cell culture

The C2C12 cells were grown in 75 cm^2 culture flask (Bedford, MA, USA) to get the enough cell count for cell seeding and subculture. Briefly, C2C12 cells were cultured in DMEM, (pH 7.4) containing 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin solution. Cells were grown and maintained in a humidified incubator at 37 °C with 5% CO_2 and 95% air environment. The cell density of 1×10^4 cells/well was seeded in a 96-well plate. Pure PPC and PPC/PLA composite nanofibrous mats were cut into square pieces (1×1 cm) and were sterilized by UV exposure, rinsed with PBS and then soaked in the medium for 2 h prior to cell seeding to facilitate protein adsorption and cell attachment. After 2 h the medium was replaced with fresh medium and culture plate was seeded with a density of 1×104 cells/well. The cells were allowed to attach and grow in wells on the nanofibrous mats for specific time (24, 48, and 72 h) duration. Myoblasts grown in the wells served as controls. The exhausted medium was replaced with fresh DMEM medium at regular intervals of time throughout the incubation period. The morphology of cultured cells and growth was regularly monitored under using a light phase contrast microscope (CX41; Olympus Corporation, Tokyo, Japan) at magnification 40×, and the photographs were taken at different time intervals by computerized color FOculus IEEE 1394 digital camera (NET New Electronic Technology GmbH, Finning, Germany) using the DIXI image solution software.

2.4. CCK-8 assay

The CCK-8 assay was performed to check the cell viability in the presence of pure PPC and PPC/PLA composite nanofibers. In brief, media from the microplates was taken out after specified incubation and replaced with fresh media in which 10 μL of water-soluble tetrazolium-8 solution in each well was added and incubated for 4 h at 37 °C according to the manufacturer's instructions. At the end of the experiment, absorbance was measured at 450 nm for each well by a microplate spectrophotometer (model 680; Bio-Rad Laboratories, Hercules, CA). Samples containing cells were taken out after incubation and were rinsed twice with PBS to remove the non-adherent cells, and subsequently fixed with glutaraldehyde (2.5%) for 4 h. After that, the samples were again rinsed with PBS and then serially dehydrated with graded ethanol concentrations for 10 min each. Finally the samples were dried and morphology was