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Poly(hydroxybutyrate)/cellulose acetate blend nanofiber scaffolds: Preparation, characterization and cytocompatibility

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ABSTRACT

Poly(hydroxybutyrate) (PHB)/cellulose acetate (CA) blend nanofiber scaffolds were fabricated by electrospinning using the blends of chloroform and DMF as solvent. The blend nanofiber scaffolds were characterized by SEM, FTIR, XRD, DSC, contact angle and tensile test. The blend nanofibers exhibited cylindrical, uniform, bead-free and random orientation with the diameter ranged from 80–680 nm. The scaffolds had very well interconnected porous fibrous network structure and large aspect surface areas. It was found that the presence of CA affected the crystallization of PHB due to formation of intermolecular hydrogen bonds, which restricted the preferential orientation of PHB molecules. The DSC result showed that the PHB and CA were miscible in the blend nanofiber. An increase in the glass transition temperature was observed with increasing CA content. Additionally, the mechanical properties of blend nanofiber scaffolds were largely influenced by the weight ratio of PHB/CA. The tensile strength, yield strength and elongation at break of the blend nanofiber scaffolds increased from 3.3 ± 0.35 MPa, 2.8 ± 0.26 MPa, and 8 ± 0.77% to 5.05 ± 0.52 MPa, 4.6 ± 0.82 MPa, and 17.6 ± 1.24% by increasing PHB content from 60% to 90%, respectively. The water contact angle of blend nanofiber scaffolds decreased about 50% from 112 ± 2.1° to 60 ± 0.75°. The biodegradability was evaluated by in vitro degradation test and the results revealed that the blend nanofiber scaffolds showed much higher degradation rates than the neat PHB. The cytocompatibility of the blend nanofiber scaffolds was preliminarily evaluated by cell adhesion studies. The cells incubated with PHB/CA blend nanofiber scaffold for 48 h were capable of forming cell adhesion and proliferation. It showed much better biocompatibility than pure PHB film. Thus, the prepared PHB/CA blend nanofiber scaffolds are bioactive and may be more suitable for cell proliferation suggesting that these scaffolds can be used for wound dressing or tissue-engineering scaffolds.

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1. Introduction

Fundamental knowledge of cell–substrate interactions is key point for tissue engineering. Topographical cues, independent of biochemistry, generated by an extracellular matrix (ECM) may have significant effects on cellular cell seeding, adhesion, proliferation and differentiation [1–4]. Thus, preparation of matrices with characteristics of natural extracellular matrix (NECM) has attracted much interest in recent years. In general, the tissue development is controlled in three matrix size scales. The gross shape and size of tissue are decided by the macroscopic shape (cm to mm scale) of matrix; cell invasion and growth are controlled by the size and structure of the matrix pore (μm); the adhesion and gene expression of cells are adjusted by the surface chemistry of the matrices (nm scale). A number of processing techniques such as particulate leaching [5,6], template synthesis [7,8], phase separation [9,10], freeze drying [11,12] and self-assembly [13,14] have been used to prepare biomimetic ECM as tissue engineering scaffolds. However, the diameters of the matrix fibers and pores are often at micron sizes and still far from the NECM which is a three-dimensional network structure composed of natural fibers ranging from 50 to 500 nm [15]. Hence, the design of scaffold composed of nanofibers has become one of the exciting new areas in tissue engineering.

Electrospinning technique provides a versatile and effective method to prepare fibers with the diameters in the range of several microns down to a few tens of nanometers. The electrospun fibrous nonwoven mats are characterized by high surface area to volume/mass ratio, high porosity, small pore size with interconnected structure, thus resembling the NECM. These characteristics have made the electrospun fibrous nonwoven mats which can be applied in membrane filtration [16,17], metal ion adsorption [18,19], optical sensors and biosensors [20,21], wound dressings [22,23], and tissue engineering [24,25].

Poly(hydroxybutyrate) (PHB) is a thermoplastic polyester produced by various microorganisms as a reserve energy source. Due to its inherent biocompatible and biodegradable properties, PHB is ideal for various biomedical applications such as controlled release system [26], surgical sutures [27], wound dressing [28], orthopedic uses [29] and dura

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substitute [30]. Preparation of electrospun PHB scaffold has become a topic of current interest. The in vitro biocompatibility studies of PHB scaffolds fabricated by solution-cast solvent and electrospinning techniques have been reported with Schwann cells [31]. The cell adhesion on the surface of PHB film scaffold is good, while those on the surfaces of PHB electrospun scaffold also appear in their characteristic spindle shape, but with the cells being able to penetrate to the inner side of the scaffolds. This result indicates that PHB electrospun scaffold is more suitable for cell proliferation and differentiation. A comparison of PHB electrospun nanofiber scaffolds with poly(e-caprolactone) (PCL), silk, poly-lactic acid (PLA), and polyamide (PA) electrospun nanofiber scaffolds for cardiac repair has been presented by Castellano. PHB electrospun nanofibers modify the inflammatory response to an M_2 macrophage phenotype in cardiac tissue, indicating PHB as a superior substrate for cardiac repair [32]. Electrospun nanofiber mats of PHB, PHBV and their blend have been fabricated and used as bone scaffolds [33,34]. In comparison with the corresponding solution-cast film scaffolds, all of the nanofibrous scaffolds exhibit much better support for cell attachment and proliferation, implying a high potential application of these electrospun PHB nanofiber mats as bone scaffolds. PHB/nanosized hydroxyapatite blend scaffolds manufactured by gas-jet/electrospinning illustrate that the electrospun scaffolds possess an extracellular matrix-like topography [35]. Biocomposite scaffolds based on electrospun PHB nanofibers and electrosprayed hydroxyapatite nanoparticles have been prepared and used for bone tissue engineering [36]. Electrospun PHB/magnetite nanofibrous nonwoven has been developed using 2,2,2-trifluoroethanol (TFE) as solvent. The degradation rate can be controlled by loading of magnetite nanoparticles [37].

Further studies show that the electrospun scaffolds have positive effects on attachment, proliferation and differentiation of bone marrow stroma cells (BMSCs). PHB/nanotholits scaffolds have also been electrospun for bone tissue regeneration [38]. PHB/chitosan based polymeric scaffolds and PHB/chitosan ultrafine fiber mats as skin regeneration prepared by electrospinning have been reported recently [39,40]. The cytotoxicity assessment with mouse fibroblast cells (L929) is investigated and the cell culture results show that electrospun PHB/chitosan scaffold benefits promoting the cell attachment and proliferation. PHB/gelatin core–shell structured electrospun fiber mats have been prepared by coaxial electrospinning. The fiber mats support the growth of human dermal fibroblasts and keratinocytes with normal morphology indicating its potential as a scaffold in tissue engineering [41]. Electrospun PHB/poly(L-lactide-co-ε-caprolactone) (PLCL) composite as nanofibrous scaffolds has been reported by Daranarong. Analysis shows that PHB/PLCL nanofibrous scaffolds can promote cell cycle progression and reduce the onset of necrosis compared to their individual PHB and PLCL components suggesting potential in the repair and engineering of nerve tissue [42].

As a well known derivative of cellulose, cellulose acetate (CA) has been used in a variety of applications such as film based photography [43], component in adhesive [44], reverse osmosis [45] and nanofiltration membrane [46]. CA fibrous structures have been produced via the electrospinning technique [47]. By now, CA/carbon nanotube blend nanofibers, CA/metal particle blend nanofibers, CA/metal oxide blend nanofibers, CA/polyacrylonitrile blend nanofibers, CA/polyvinyl alcohol blend nanofibers and CA/chitosan blend nanofibers have been fabricated and shown potential applications in biomedical, tissue engineering, sensor, adsorbent, etc. [48–55].

In this paper, we are reporting the preparation of poly(hydroxybutyrate) (PHB)/cellulose acetate (CA) blend nanofiber scaffolds by electrospinning technique using chloroform/DMF as co-solvent. The blend nanofiber scaffolds were characterized by SEM, FTIR, DSC, XRD, water contact angle and tensile test. The biodegradability and cytocompatibility of the PHB/CA blend nanofiber scaffolds were also preliminarily evaluated by in vitro degradation test and cell attachment studies. The present contribution aims at combining these two biodegradable and biocompatible polymers to fabricate new blend nanofiber scaffolds with potential application in biomedical field.

2. Materials and methods

2.1. Materials

The poly(hydroxybutyrate) (PHB), a white powder sample, was kindly provided by Tianjin TianLu Co. Ltd. (China), $Mw = 4.3 \times 10^5$ (obtained by G.P.C. in chloroform at 30 °C). CA with an acetyl content of 40% was purchased from Fluka. Chloroform and other chemicals of the highest purity available were used and purchased from Sigma-Aldrich, USA.

2.2. Preparation of PHB film

PHB was dissolved in chloroform/DMF (70:30 v/v) to make a 5% solution. Then, the transparent PHB solution was spin-coated on wafer and dried at room temperature for 12 h. To ensure complete elimination of the solvent, the films were then dried at 60 °C for 6 h. The PHB films were obtained by peeling them off from the wafer.

2.3. Preparation of PHB/CA blend nanofiber scaffolds

The production parameters for PHB/CA blend nanofiber scaffolds were summarized in Table 1. Electrospinning solutions were prepared at several PHB/CA ratios (100:0, 90:10, 80:20, 70:30, 60:40 and 0:100 w/w), 5% total polymer, in blends of chloroform/DMF (70:30 v/v). Electrospinning of PHB/CA blend solutions was carried out as the following procedures. PHB/CA blend solution was filled into a glass syringe terminated by a stainless steel needle with inner diameter of 0.50 mm. The syringe was placed in an automatic pump and PHB/CA blend solution was extruded out at a constant speed of 0.2 ml/h. High voltage ranging from 24 to 30 kV was applied in the electrospinning process. The tip-to-collector distance was fixed at 25 cm. The PHB/CA blend nanofibers were collected by Al foil. The experiment was done in an environmental chamber with constant temperature at 25 °C and the relative humidity (RH) of 35%.

2.4. Porosity measurements

The porosity of scaffold was estimated using Archimedes' principle based on fluid displacement measurement techniques [56]. Briefly, individual scaffolds (disc-shaped: 10 mm diameter, 5 mm thickness) were placed in a graduated cylinder filled with a known volume of ethanol ($V1$). The total volume following scaffold immersion ($V2$) was recorded. After 1 h, the scaffolds were removed with the entrapped solvent in the pores, and the volume of ethanol left in the cylinder was denoted by $V3$. The total volume (VT) of the scaffolds was determined using the equation $VT = V2 - V3$. The porosity was then determined by calculating

Table 1
Production parameters for PHB/CA blend nanofiber scaffolds.

PHB/CA ratios	Solution concentration (%)	Extrusion speed (ml/h)	Applied voltage (kV)	Reception distance (cm)
100/10	5	0.2	24	25
90/10	5	0.2	24	25
80/20	5	0.2	26	25
70/30	5	0.2	28	25
60/40	5	0.2	30	25
0/100	5	0.2	24	25

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