



Incorporation of poly(ethylene glycol) grafted cellulose nanocrystals in poly(lactic acid) electrospun nanocomposite fibers as potential scaffolds for bone tissue engineering



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ARTICLE INFO

Article history:

Received 22 September 2014

Received in revised form 9 November 2014

Accepted 6 January 2015

Available online 8 January 2015

Keywords:

Poly(lactic acid)

Cellulose nanocrystals

Poly(ethylene glycol)

Interfacial adhesion

Mechanical properties

ABSTRACT

Poly(ethylene glycol) (PEG)-grafted cellulose nanocrystals (CNCs) were successfully synthesized and incorporated into poly(lactic acid) (PLA) as a reinforcing filler to produce nanocomposite scaffolds consisting of CNC-g-PEG and PLA using an electrospinning technique. Morphological, thermal, mechanical, and wettability properties as well as preliminary biocompatibility using human mesenchymal stem cells (hMSCs) of PLA/CNC and PLA/CNC-g-PEG nanocomposite scaffolds were characterized and compared. The average diameter of the electrospun nanofibers decreased with increased filler loading level, due to the increased conductivity of the electrospun solutions. DSC results showed that both the glass transition temperature and cold crystallization temperature decreased progressively with higher CNC-g-PEG loading level, suggesting that improved interfacial adhesion between CNCs and PLA was achieved by grafting PEG onto the CNCs. Wettability of the electrospun nanofibers was not affected with the addition of CNCs or CNC-g-PEG and indicating that the fillers tended to stay inside of the fiber matrix under electrical field. The tensile strength of the composite fiber mats was effectively improved by the addition of up to 5% CNC-g-PEG up to 5 wt.%. In addition, the cell culture results showed that PLA/CNC-g-PEG composite nanofibers exhibited improved biocompatibility to hMSCs, which revealed the potential application of this nanocomposite as the scaffolds in bone tissue engineering.

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1. Introduction

Cellulose is the world's most abundant renewable polymer resource and has been used as an engineering material for thousands of years [1]. By extracting cellulose at the nanoscale, the majority of the defects associated with its hierarchical structure can be removed, and a new generation of material–cellulose nanoparticles can be obtained, which is an ideal material on which to base the new biopolymer nanocomposite industry. Crystalline cellulose has a greater axial elastic modulus than Kevlar and its mechanical properties are within the range of other reinforcement materials [2,3]. The preparation of reinforced polymer materials with cellulose nanoparticles has seen rapid advances and considerable interest in the last decade owing to its renewable nature, high mechanical properties, and low density, as well as its availability and the diversity of its sources. Cellulose nanocrystals (CNCs) are defect-free, rod-like crystalline residues obtained when cellulose is subjected to acid hydrolysis. CNCs have attracted great attention due to

their high aspect ratio (3 to 5 nm wide, 50 to 500 nm in length) and high crystallinity (54 to 88%). It has been reported that CNCs can be aligned under high electrostatic fields [4]. During the electrospinning process of polymer/CNC nanofibers, CNCs orient along the fiber axis, which endows electrospun nanofibers with significantly enhanced axial strength [5,6].

Poly(lactic acid) (PLA) has been historically employed in the biomedical and tissue engineering fields in applications such as resorbable sutures, antibiotic release materials, and degradable implants due to its bioresorbable and biocompatible properties [7]. PLA-based cellulose nanocrystal composites have been extensively investigated in recent years to develop the next generation of lightweight and high performance materials for biomedical applications [8–11]. Among different bio-fabrication methods, electrospinning has been widely used to produce porous PLA-based scaffolds for tissue engineering applications due to its simple setup and its ability to generate fibers with diameters ranging from 50 nm to a few micrometers and a fiber mat with high interconnectivity and high surface areas that resembles natural extracellular matrix (ECM) [12–15]. It has been found that the physiological characteristics of the scaffolds such as mechanical properties can be

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tailored to control the cell behavior. However, the poor compatibility related to the interfacial adhesion between the hydrophilic CNCs and the hydrophobic PLA matrix hindered the nanoparticles from dispersing well in the matrix. Therefore, it has become increasingly important to improve the dispersion of CNCs in the PLA matrix to enhance the mechanical properties of such composites and improve the commercial viability of such products as scaffolds in tissue engineering. To overcome this challenge, surface modification of cellulose by partial substitution of hydroxyl groups with other functional groups seems to be an effective strategy. From the literature review, methods of surface modification of cellulose nanocrystals can generally be categorized into three distinct groups: (1) substitution of surface hydroxyl groups with small molecules [16], (2) polymer surface modification with different coupling agents [17,18], and (3) polymer surface modification with radical polymerization [19–21].

To facilitate the dispersal of CNCs in hydrophobic systems, as well as to achieve steric colloidal stabilization of CNCs, Grey et al. reported the method of surface grafting of cellulose nanocrystals with poly(ethylene glycol) (PEG) [18]. PEG is water soluble and also can be dissolved in hydrophobic solvents such as chloroform. By grafting the surface hydroxyl groups of CNCs with PEG, the polymer chains extend into the surrounding aqueous medium like “polymer brushes,” thus hindering direct contact between the nanoparticles and therefore inhibiting coagulation of the suspension. The monofunctionalized PEG with an epoxide end group can react with the hydroxyl groups on the surface of the CNCs in alkali aqueous media. Nucleophilic attack by surface hydroxyl groups under strongly alkaline reaction conditions opens the epoxide ring to form a covalent ether linkage between the CNCs and PEG chains.

While CNC-g-PEG has been synthesized and reported in the literature [18], to the best of our knowledge, this modified form of CNC has not been incorporated into PLA-based composites to improve the compatibility between CNC and PLA and to enhance the mechanical properties of the resulting nanocomposite fibers. In addition, the PEG with an epoxy end group used in the previous study had a molecular weight of 2.086 kDa. In this study, the hydroxyl groups present on the surface of the cellulose nanocrystals were partially substituted with PEG. The PEG epoxide (PEG-EP) was synthesized from poly(ethylene glycol) hydroxyl (PEG-OH). PEG ($M_n = 5000$) was then grafted onto the CNCs. By grafting higher molecular weight of PEG onto CNCs, we have achieved longer “polymer brushes” on the surface of CNC. It is hypothesized that the grafting of CNCs with PEG will improve the dispersion of CNCs in organic solvents. It is further hypothesized that the addition of CNC-g-PEG will promote miscibility and interfacial adhesion with the PLA matrix, ultimately enhancing the mechanical performance of the resultant composites. The successful monofunctionalization of PEG-OH with an epoxide end group, and the further grafting onto CNCs, was confirmed by proton nuclear magnetic resonance (^1H NMR) spectra and Fourier transform infrared spectroscopy (FTIR). Bionanocomposite nanofibers consisting of CNC-g-PEG and PLA were fabricated using the electrospinning technique. The composite nanofibers were characterized using scanning electron microscopy (SEM), differential scanning calorimetry (DSC), water contact angle (WCA) testing, and tensile measurements. The formation of our bones involves the progeny of human mesenchymal stem cells (hMSCs) which can be differentiated into osteoblast-like cells [22]. Preliminary biocompatibility assay of PLA/CNC-g-PEG nanocomposite scaffold was investigated with hMSCs to explore its potential application in bone tissue engineering.

2. Experimental setup

2.1. Preparation of cellulose nanocrystals (CNCs)

Commercially available dissolving pulp dry lap made from southern pine was used as the starting material for producing cellulose nanocrystals (CNCs). Strips of the dissolving pulp were reacted with

64% sulfuric acid at 45 °C for approximately 1.5 h under a nitrogen blanket with constant stirring. The hydrolysis reaction was terminated by diluting with water to approximately a 10-fold volume of the initial suspension. The CNCs were neutralized by adding an aqueous sodium hydroxide solution of about 5% concentration. At this point, the CNCs were not colloidal and settled because of the high salt concentration. Continued dilution and decanting were carried out until the sodium sulfate concentration was about 1%. The sodium sulfate and other salts were removed by ultrafiltration in a tubular ultrafiltration unit. Fresh water was added to the suspension during ultrafiltration to keep the CNC concentration at approximately 1%. After essentially all of the salt was removed, the CNC suspension was concentrated in the same ultrafiltration unit by no longer adding fresh water. The final concentration of the CNCs was approximately 10% by weight.

2.2. Synthesis of poly(ethylene glycol) epoxide (PEG-EP)

Poly(ethylene glycol) epoxide (PEG-EP) with an epoxy end group was synthesized from poly(ethylene glycol) hydroxyl (PEG-OH) in two steps following the procedure described in the literature [23]. First, 10 g of PEG-OH (M_n 5000) and 0.05 g sodium were stirred in 80 ml toluene at 100 °C for 8 h under nitrogen flow and reflux water. The solution was then cooled down to 40 °C and 0.3 g epibromohydrin was added and further stirred for 12 h. PEG-EP was obtained after the solution was filtered, washed twice with diethyl ether, and freeze dried.

2.3. Poly(ethylene glycol) (PEG) grafting reaction

The grafting reaction of poly(ethylene glycol) epoxide onto cellulose nanocrystals was performed as follows. Briefly, NaOH aqueous solution (0.62 g in 12 ml deionized water) was added dropwise to a diluted CNC suspension (30 g, 2.22% w/w) to give a total concentration of 0.37 mol/l NaOH. The mixture was stirred for 30 min before 1.03 g of PEG-EP was added. The mixture was heated to 65 °C and stirred for 6.5 h followed by exhaustive dialysis against DI water (dialyzed for 1 week in a 5 l container; water was changed 5 times a day) to remove any NaOH and unreacted PEG. Poly(ethylene glycol) grafted cellulose nanocrystal (CNC-g-PEG) powder was obtained after the dialyzed solution was freeze dried.

2.4. Fabrication of electrospun nanofibers

Poly(lactic acid) (PLA, 2002D, D content of 4.25 wt.%, melt flow index of 7 g/10 min, density of 1.24 g/cm³) was purchased from NatureWorks, LLC (Minnetonka, MN, United States). N,N'-dimethylformamide (DMF) and chloroform were purchased from Sigma-Aldrich Inc. (St. Louis, MO, United States). PLA was dissolved in 7 ml of chloroform with magnetic stirring for 3 h at room temperature. CNC-g-PEG was added to 3 ml of DMF and ultrasonic treated for 10 min. The two solutions were mixed together and further stirred for 6 h before electrospinning. The weight concentration of PLA in the mixture was fixed at 10%, and the loading levels of CNC-g-PEG by weight of PLA were 1, 5, and 10%. Pure CNCs were also added to PLA following the same procedure at a 1 and 5% content because the dispersion of CNCs in electrospun PLA nanofibers weakens above a 5% CNC loading level [12]. The nomenclature of the samples was designated as PLA/CNC (x%) and PLA/CNC-g-PEG (x%), where x was the weight percent of the fillers in relation to PLA. The PLA solution, and PLA/CNC and PLA/CNC-g-PEG suspensions prepared, were separately loaded into a 5 ml plastic syringe with an 18 gauge blunt stainless steel needle and then mounted on a syringe pump. The flow rate of the suspension was controlled at 0.5 ml/h. The fibers were electrospun onto a piece of aluminum foil within a 20 kV electric field at a 15 cm needle tip-to-collector distance. The obtained nanofibers were dried under vacuum at 80 °C for 24 h and then stored in a desiccator prior to characterization.

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