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Development of tannic acid/chitosan/pullulan composite nanofibers from aqueous solution for potential applications as wound dressing

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ABSTRACT

This study presents the successful development of biocompatible tannic acid (TA)/chitosan (CS)/pullulan (PL) composite nanofibers (NFs) with synergistic antibacterial activity against the Gram-negative bacteria *Escherichia coli*. The NFs were developed utilizing the forcespinning® (FS) technique from CS-CA aqueous solutions to avoid the usage of toxic organic solvents. The ternary nanofibrous membranes were crosslinked to become water stable for potential applications as wound dressing. The morphology, structure, water solubility, water absorption capability and thermal properties of the NFs were characterized. The ternary composite membrane exhibits good water absorption ability with rapid uptake rate. This novel membrane favors fibroblast cell attachment and growth by providing a 3D environment which mimics the extracellular matrix (ECM) in skin and allows cells to move through the fibrous structure resulting in interlayer growth throughout the membrane, thus favoring potential for deep and intricate wound healing.

1. Introduction

Nanofiber (NF) based structures have shown positive results as three dimensional (3D) scaffolds for cell adhesion and proliferation (Bolin et al., 2009). These artificial 3D nanofibrous structures feature a morphological similarity to the natural extracellular matrix (ECM) in skin, which is characterized by a wide range of pore sizes, high porosity and high mechanical endurance (Kumbar, James, Nukavarapu, & Laurencin, 2008; Pham, Sharma, & Mikos, 2006). Therefore, nanofibrous structures have immense protential as tissue engineering scaffolds for skin substitutes (Min et al., 2004; Rieger, Birch, & Schiffman, 2013), which favor cell adhesion, migration and proliferation (Mattioli-Belmonte et al., 2007). In the last few decades, electrospinning has been the most widely used method to prepare fine fibers (Paneva, Bougard, Manolova, Dubois, & Rashkov, 2008). Recently, forcespinning® (FS) has proven successful as a viable and versatile method to mass produce polymeric fibers. Unlike electrospinning, which draws fibers through the use of electrostatic forces, FS utilizes centrifugal forces which allow for a significant increase in yield, ease of production, and a broader choice of materials to be spun as fibers (Padron, Fuentes, Caruntu, & Lozano, 2013). Both conductive and non-conductive polymer solutions and polymer melts can be spun into fibers without the need of electric fields (Lozano and Sarkar, 2009; Sarkar et al., 2010). As reported, the productivity of this method (over 1 g min^{-1} per nozzle) at the lab scale is significantly higher than lab scale electrospinning (0.3 g h^{-1}) (Ramakrishna, 2005).

Chitosan, the linear cationic (1-4)-2-amino-2-deoxy-β-D-glucan with typical degree of acetylation ca. 0.25, is soluble in certain acidic aqueous solutions, owing to the protonation of the primary amino groups. Chitosan lends itself to a number of chemical reactions, including thermally induced amide formation. All derivatives keep the inherent filmogenicity and antimicrobial activity of plain chitosan. In all fields of study relevant to the administration of chitosan to the human body, chitosan exhibits favorable actions, including immunostimulating activity (Jeong, Kim, & Kim, 2013; Muzzarelli, 2010, 2012; Muzzarelli et al., 2012; Muzzarelli et al., 1990; Qu, Wirsén, & Albertsson, 1999a,b; Ravi Kumar, Muzzarelli, Muzzarelli, Sashiwa, & Domb, 2004). However, due to the strong intermolecular interaction of CS macromolecules, it is difficult to directly spin pure chitosan (Ohkawa, Cha, Kim,

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Nishida, & Yamamoto, 2004). In order to improve the fiber forming ability of CS and expand its potential applications, CS is commonly blended with other polymers that possess fiber forming capabilities such as polyethylene oxide (PEO) (Desai, Kit, Li, & Zivanovic, 2008; Pakravan, Heuzey, & Ajji, 2011), polyvinyl alcohol (PVA) (Jia et al., 2007; Ohkawa et al., 2004), polylactic acid (PLA) (Xu et al., 2009), silk fibroin (Park, Jeong, Yoob, & Hudson, 2004) and collagen (Chen, Mo, & Qing, 2007), which are all biocompatible and biodegradable and will not restrict the final applications of CS NFs.

Pullulan (PL) is a neutral linear, fungal exopolysaccharide consisting of α-1,6-linked maltotriose residues, produced from starch by *Aureobasidium pullulans* (Yuen, 1974). Besides the common advantages of natural polysaccharides, such as non-toxic, biocompatible and biodegradable, the distinctive linkage pattern of PL endows it with unique physical properties, including excellent water solubility, high-water-absorbing capability, adhesive properties and the capability to form strong resilient films and fibers (Li et al., 2011; Singh, Saini, & Kennedy, 2008). The PL films and fibers are edible, colorless, tasteless, odorless, flexible, and possess excellent mechanical properties (Kristo, Biliaderis, & Zampraka, 2007). PL can be easily molded and has been proven effective for fiber formation (Singh et al., 2008). Recently PL is being investigated for several biomedical applications such as targeted drug and gene delivery (Rekha & Sharma, 2009), tissue engineering (Na, Shin, Yun, Park, & Lee, 2003), wound dressing (Li et al., 2011) to mention some.

Tannic acid (TA) is a gallic ester of D-glucose in which the hydroxyl groups of the carbohydrate are totally esterified with gallic acid dimers. Its multiple phenolic groups can interact with biological macromolecules (Aelenei, Popa, Novac, Lisa, & Balaita, 2009). TA possesses strong astringent, antioxidant, hemostatic and antibacterial properties and therefore besides others uses in the textiles, wine, and wood industries, it has found beneficial applications in the medical area as a drug for the treatment of skin ulcers, burns, wounds and toothache. It is expected that the addition of TA to other biomaterials will synergistically enhance healing properties.

In this study, we mass produced CS/PL binary composite NFs from a CS citric acid (CA) aqueous solution using FS technique. The aqueous solution was applied not only to avoid the trace presence of the toxic organic solvents in the produced fibers but also to promote the development of functional fibers containing water-soluble drugs for wound dressing applications. The drug, TA was then incorporated fabricating water-stable TA/CS/PL ternary composite NFs through subsequent crosslinking. The morphology, structure, water solubility, water absorption capability, and thermal properties of the NFs were characterized. The antibacterial activity of the ternary composite NFs against gram-negative bacteria, *Escherichia coli* was investigated. The biocompatibility and the potential applications of these fiber membranes as wound dressing was evaluated in vitro with mouse embryonic fibroblasts (NIH 3T3).

2. Experimental

2.1. Materials

Pullulan was purchased from Tokyo Chemical Industry Co. (Japan). Low molecular weight Chitosan (M_w = 50,000–190,000 and 75–85% degree of deacetylation), tannic acid (produced from Chinese natural gall nuts) and citric acid were purchased from Sigma–Aldrich. Anhydrous potassium bromide (KBr, >99%) was purchased from Fisher Sci. Deionized water (DI water, 18 MΩ cm) was produced from Mill-Q (Millipore Ltd., UK).

2.2. Preparation of the solutions

The 3 wt% CS-CA salt solution was prepared by dissolving chitosan in citric acid aqueous solution, and then allowing the solution to stir overnight. PL (18 wt%) was added into CS-CA solution and continuously stirred at room temperature until the solution became clear. TA (1 wt%) was then added into the solution, and stirring continued until it became clear.

2.3. Production of NFs

FS was performed on a lab scale Cyclone™ L-1000M (manufactured by FibeRio Technology, Corp.). Work reported in the literature has shown the schematics of the FS system as well as detailed description of the theory and experimental processes (Padron et al., 2012, 2013; Raghavan, Soto, & Lozano, 2013; Sarkar et al., 2010; Weng, Xu, & Garza et al., 2014; Weng, Xu, & Lozano, 2014; Weng, Xu, Salinas, & Lozano, 2014). A cylindrical spinneret equipped with 30 gauge half-inch regular bevel needles (Becton, Dickinson and Company) was selected; 2 mL of the prepared polymer solution were injected into the spinneret using a 5-mL plastic syringe. The polymer solution was extruded through the orifices by centrifugal force and fiber jets were formed. Fibers were collected either by utilizing a deep-dish fiber collector with equally distanced vertical pillars or by allowing the fibers to deposit on a polypropylene substrate. After collection, the fibers were covered and stored under desiccation.

2.4. Crosslinking of the FS membranes and stability test

The TA/CS/PL composite membranes were cured in an oven at 150 °C for at least 1 h to induce chemical crosslinking with CA. After crosslinking, the composite NFs were immersed in DI water for 48 h. Then the samples were dried under ambient conditions prior to analysis.

2.5. Characterizations of NFs

A field emission scanning electron microscopy (FE-SEM; Sigma VP Carl Zeiss, Germany) was used to analyze fiber morphology, homogeneity, orientation, and fiber size. The average diameter of the NFs was analyzed by randomly measuring 300 different NFs from the SEM images using the image analysis software (JMicroVision V.1.2.7, University of Geneva, Geneva, Switzerland).

Fourier transform infrared spectroscopy (FTIR) measurements were performed on a Bruker IFS 55 Equinox FTIR spectrometer. The FTIR spectra were recorded from KBr pellets in the range of 4000–400 cm^{-1}.

X-ray diffraction (XRD) studies were conducted on a Bruker-AXS D8 advance X-ray diffractometer using a Cu Kα radiation source filtered with a graphite monochromator (λ = 1.5406 Å). ^1H nuclear magnetic resonance (NMR) measurement was carried out on a Bruker DRX600 600 MHz nuclear magnetic resonance spectrometer at 25 °C. 10 mg of the ternary composite NF membrane before crosslinking were dissolved in 0.75 mL deuterium oxide and loaded into the NMR rotor at external magnetic field strengths of 14.1 T.

Thermo-physical analysis was conducted through thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC). Both TGA and DSC instruments are from the TA-Q series, model TGA-Q500 and DSC-Q100 (TA Instruments Inc.), respectively. For TGA, 10 mg samples were heated on platinum pans from room temperature to 600 °C at a heating rate of 5 °C min^{-1} under air and nitrogen flow (30 mL min^{-1}). For DSC, samples of about 10 mg were sealed in aluminum pans and heated from room temperature to 200 °C at a heating rate of 1 °C min^{-1} under 30 mL min^{-1} of air flow, and then cooled to room temperature again at the same rate.