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Research Paper

O-acylation of chitosan nanofibers by short-chain and long-chain fatty acids

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ABSTRACT

Chitosan nanofibers (CSNFs) have potential applications in biomaterials, oil recovery and food packaging, but their instability in moist environment has limited their full utilization. Here we report that CSNFs can be O-acylated in a post-electrospinning treatment by using pyridine as catalyst and short-chain (C2, C3, C4, C5 and C6) and long-chain (C8 and C12) fatty acid anhydrates as acylation agents. The effects of O-acylation to CSNFs were analyzed in detail. FT-IR, ^{1}H NMR and elemental analysis indicated that the hydroxyl groups of chitosan in CSNFs were acylated in 2 h. XRD spectra indicated that the O-acylation modification altered the crystal structure of the native fibers and the acyl substituents packed in a laterally aligned and layered structure. SEM examinations showed that the acylation modification could effectively control the fibrous structure of CSNFs and improve their stability in moist environment. The O-acylated CSNFs generally have an average diameter about 100 nm except for laurelated CSNFs (~200 nm). Water contact angle measurement indicated that the wetting properties of O-acylated CSNFs were affected by the length of acyl side chains. This fiber acylation strategy can tune the material properties of CSNFs and expand their potential applications.

1. Introduction

Chitosan is the N-deacetylated product of chitin, which is the second most abundant polysaccharide in nature and widely exists in the shell of crustaceans such as crabs and also exists in the cell wall of fungi such as yeast(Bano, Arshad, Yasin, Ghauri & Younus, 2017; Klis, Boorsma & De Groot, 2006). Chemically, chitosan is a linear polysaccharide that the monomers are randomly distributed D-glucosamine units and N-acetyl-D-glucosamine units. Chitosan have many structural characteristics similar to glycosaminoglycans such as hyaluronic acid and have many favorable biological properties such as anti-bacterial, biocompatible, biodegradable, anticoagulant and wound healing effects, which are of great interest in biomedical applications(Cosco et al., 2016; Riteau & Sher, 2016; Taranejoo, Chandrasekaran, Cheng & Hourigan, 2016; Xia et al., 2016). Chitosan have been fabricated in the form of electrospun nanofibers, core-shell nanoparticles, stimuli-responsive hydrogels and other structures (Liu, Chen, Liu & Liu, 2008; Min et al., 2004; Rinaudo, 2006). However, its exploitation in biomedical field is somewhat limited by its poor aqueous stability. Chemical modification of chitosan nanomaterials can be an effective strategy to improve their structural stability and expand their applications.

Acylation modification of polysaccharide based nanomaterials is an important strategy to change their surface polarity and increase their dimensional stability, resulting in an increased number of potential applications(Avila Ramirez, Gomez Hoyos, Arroyo, Cerrutti & Foresti, 2016; Kim, Nishiyama & Kuga, 2002; Yuan, Nishiyama, Wada & Kuga, 2011). Among various polysaccharide nanomaterials, electrospun CSNFs have received a great deal of attention in many fields such as wound dressings, tissue engineering, drug delivery, filtration and biosensors(Lee, Jeong, Kang, Lee & Park, 2009). Most of the previously reported CSNFs were mixed with additive polymers such as PEO or PVA, as a fiber forming aid, resulting in an undesirable chemical heterogeneity of the fibers and making further chemical modifications troublesome(Huang, Ge & Xu, 2007; Jayakumar, Prabaharan, Nair & Tamura, 2010; Pakravan, Heuzey & Ajji, 2012). Additive-free CSNFs can be prepared by electrospinning of chitosan solubilized in the mix solvents of trifluoroacetic acid and dichloromethane(Ohkawa, Cha, Kim, Nishida & Yamamoto, 2004). The resultant CSNFs have a very homogenous structure without beads and branches. However, the resultant CSNFs suffered from extensive swelling due to the high surface polarity of the fibers (And & Schauer, 2007; Cooper, Bhattarai, Kievit, Rossol & Zhang, 2011; Zomer, Almodóvar, Erickson, Popat,

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Migliaresi & Kipper, 2012).

Chemically modified CSNFs have been found to possess better stability than unmodified CSNFs in a number of studies(Cooper, Bhattarai, Kievit, Rossol & Zhang, 2011; Zomer et al., 2012). Chitosan derivative nanofibers could be obtained by directly electrospinning of chitosan derivatives or by post-electrospinning modifications on the as-spun CSNFs. Lactic acid modified chitosan (LA-CS) nanofibers were prepared by post-electrospinning thermo treatment on the nanofibers consisting of lactic salt of chitosan, and the modified fibers exhibited positive effects on nerve regeneration with significantly improved fiber stability (Cooper et al., 2011). Directly electrospinning of chitosan derivatives such as chitosan butyrate or chitosan hexanoate were also reported. The chitosan hexanoate fibers exhibited an ununiform distribution of fiber diameter (0.64–3.93 μm) and poor fiber structure(Neamnark, Rujiravanit & Supaphol, 2006). The chitosan butyrate nanofibers showed an average diameter about 0.3 μm, but giant fibers about 1 μm in diameter were also widely present(Du & Hsieh, 2009). As we found previously, directly electrospinning of the polysaccharides derivatives with long side chains could be very difficult because these side chains would sterically hinder the formation of uniform and fine fibers(Wu et al., 2016). As far as we know, there has been no systemic study on the post-electrospinning acylation of CSNFs using a series of short-chain and long chain fatty acids, although acylation was a commonly used chemical modification for polysaccharides. Recently, esterification of bacterial cellulose nanofibers by varied methods have been widely reported and many new applications were exploited(Gonçalves et al., 2016; Ramírez, Hoyos, Arroyo, Cerrutti & Foresti, 2016). Give the similar structure of bacterial cellulose nanofibers and CSNFs, It is reasonable to expect that the properties and applications of electrospun CSNFs can also be benefited from acylation modification.

In this study, a post-electrospinning O-acylation modification was performed on the as-spun CSNFs. Mild reagents including pyridine and acetic anhydride, propionic anhydride, butyric anhydride, valeric anhydride, hexanoic anhydride, octanoic anhydride and lauric anhydride were used to obtain a series of O-acylated CSNFs. The material characteristics of O-acylated CSNFs, including their chemical structure, fiber morphology, water contact angle and crystallinity properties, were thoroughly characterized by a serious of experiments.

2. Materials and methods

2.1. Materials

Chitosan (80% deacetylated, medium viscosity, 200–400 mPa.s, $M_w = 1.9 \times 10^5$) was purchased from aladdin Inc (Shanghai, China); trifluoroacetic acid, trifluoroacetic anhydride, pyridine and acid anhydrides(acetic anhydride, propionic anhydride, butyric anhydride, valeric anhydride, hexanoic anhydride, octanoic anhydride, and lauric anhydride) were purchased from local chemical companies.

2.2. Preparation of CSNFs by electrospinning

The electrospinning method of chitosan was based on a previously published work(Ohkawa et al., 2004). The electrospinning solution was prepared by mixing 500 mg chitosan, 8 mL trifluoroacetic acid and 2 mL dichloromethane in room temperature for 8 h. The electrospinning solution was loaded into a 10 mL syringe with a stainless steel 10-gauge needle tip. The electrospinning voltage was set to 25 kV and the distance between collector and needle tip was 20 cm. The fiber was collected on a rotating stainless steel disk and typically a ~200 μm thick, round shaped (20 cm in diameter) CSNFs membrane were obtained by electrospinning the solution for 3 h. The obtained membranes were dried at 50 °C in a vacuum oven.

2.3. O-Acylation of the chitosan nanofibers

O-Acylation of CSNFs was performed according to our previous work with minor modifications(Wu, Chu, Kuang, Meng, Wang & Tang, 2013). 20 mg of as-prepared CSNFs were placed in a 10 mL glass vial containing pre-mixed solution of 1 mL pyridine and 1 mL carboxylic acid anhydride (for lauric anhydride, 500 mg of powder dissolved in 1 mL pyridine before reaction). The mixture was stirred gently at 50 rpm for 2 h at 80 °C. After acylation, the nanofibers were washed repeatly by an excessive amount of distilled water and methanol to remove the unreacted reagents and allow the hydrolysis of trifluoroacetic salts. The nanofibers were then dried by lyophilization.

2.4. Fourier transform infrared spectroscopy (FT-IR)

FT-IR spectra of the O-acylated CSNFs were recorded with a Nicolet 6700 spectrometer using KBr pellet method and all of the spectra were obtained at a resolution of 4 cm^{-1} and with a total of 32 scans with a wave number range between 500 and 4000 cm^{-1}.

2.5. Proton nuclear magnetic resonance (^1H NMR)

^1H NMR spectra were recorded using a Bruker AVANCE DRX-500 at 25 °C with a resonance frequency of 500 MHz. Trifluoroaceticacid-d was used as deuterated solvent for all the tested samples.

2.6. Elemental analysis and the estimation of degree of substitution

According to previous studies with minor modifications(Ifuku, Morooka, Morimoto & Saimoto, 2010; Zong, Kimura, Takahashi & Yamane, 2000), the content of *C*, and *N* in the aclylated CSNFs were measured using a Vario EL III (ELEMENTAR Inc.). Before analysis, the samples were vacuum dried at 60 °C overnight. The degree of substitution for the acyl groups were estimated from the elemental analysis data based on the equation below:

$$\frac{C}{12} : \frac{N}{14} = \frac{6 + px}{1}$$

Where *C* and *N* are weight percentage of carbon and nitrogen, *x* is the degree of substitution value of the acyl groups and p is the carbon number of the substituted acyl groups (specifically, p value for acetyl, propionyl, butyryl, valeryl, hexanoyl, octanoyl and lauryl groups is 2, 3, 4,5,6,8, 12 respectively).

2.7. Scanning electron microscope (SEM)

The morphology and diameter of CSNFs and O-acylated CSNFs were examined via a Hitachi S-2380N scanning electron microscope. Before SEM analysis, the samples were coated with an approximately 10 nm layer of gold by an ion sputter coater. The diameter of the obtained nanofibers was manually measured in the SEM images using Image J (NIH, v1.47) and about 50 nanofibers for each sample were analyzed.

2.8. Water contact angel measurement

Water contact angles on different O-acylated CSNFs were determined using a VCA Optima surface analysis system (Billerica, USA). The testing procedure was modified form a previously published work (Xu et al., 2010). Briefly, 5 μL of water droplets were placed carefully onto the surfaces of the nanofibers for five different positions of the same sample, and the photographs of the droplets were recorded by a digital camera. The contact angles were calculated by the goniometry software of VCA OptimaXE.