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Physico- and bio-activities of nanoscale regenerated cellulose nonwoven immobilized with lysozyme

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ABSTRACT

Lysozyme-cellulose conjugates are of wide interest for food packaging, tissue scaffolding, wound healing, and antimicrobial applications. Here a recycled cotton-based source of regenerated cellulose in combination with carboxylated carbon nanotubes and graphene oxide was configured as nonwoven nanofibrous mats through electrospinning and utilized to immobilize lysozyme. Scanning electron microscopy, Fourier transform-infrared spectra, thermal-gravimetric analysis, tensile test, and antibacterial assessments were conducted to characterize and determine physical and bioactive properties of the nonwoven nanofibrous mats. The resulted cellulose-lysozyme conjugates were found to have robust bioactivity with no indication of cell cytotoxicity. The study confirmed that the carbon-nanoparticle-modified cellulose nonwoven mats revealed a high antimicrobial activity after immobilization of lysozyme.

1. Introduction

Lysozyme is a model enzyme that breaks down the cell wall of bacteria by catalyzing hydrolysis of the 1, 4-β-glyosidic bond [1]. Since its discovery by Alexander Flemming [2], lysozyme has been extensively studied as an enzyme and antimicrobial polypeptide [3,4], and utilized as a model for enzymatic functions including the fields of applied biocatalysis and bioprocess. Lysozyme is also widely used in fine-chemical synthesis, biomedical engineering, biosensor applications and food protection [5–7]. To produce bioactive materials and improve enzyme stability and durability, enzyme immobilizations on supporting matrices have been generally studied [8,9]. In addition, immobilized enzymes are advantageous for commercial applications because of low cost, easy separation from the reaction mixture, and reusability [10].

Nanomaterials are one kind of excellent supporting materials for enzyme immobilization because of their large surface areas that help increase enzyme loading efficiency [11,12]. Typical nanomaterials used for enzyme immobilization include silica nanotubes [13], nanoporous materials [14], magnetic nanoparticles [15], and polymer nanomaterials [16].

The potential to use protein derivatives of cellulose nanofibers in tissue engineering and wound healing applications is based on their high specific surface area, biocompatibility and ease of derivatization of cellulose. This is especially the case with lysozyme-cellulose conjugates that are of interest to confer antimicrobial activity to tissue scaffolding and wound healing materials [17,18]. In addition, cellulose immobilized lysozyme shows better stability compared to free lysozyme [19,20].

Cellulose-based biomaterials including regenerated cellulose fibers are ideal support materials for enzyme immobilization because of their unique polysaccharide structure, biodegradability, biocompatibility, and non-toxicity [21–23]. These properties are important for cell protein survival [24]. It was reported that varieties of cellulose-based fibers are commonly used as protein support [25–27] among which cotton is a mostly used cellulose fiber. Cotton cellulose recycled from waste cotton fabrics are environmental friendly, abundant, and highly pure. Ionic liquid solvents and electrospinning are enabling technologies for producing regenerated cellulose micro/nano fibers and multi-functional nonwoven mats with fabrication flexibility and cost effectiveness [19]. Recently many researchers have described approaches to immobilizing lysozyme on cotton and cellulose-based textiles [20], nanocrystals [28,29] and nanofibers [30,31]. It has been found that immobilized lysozyme can confer high antimicrobial activity while sustaining resistance to degradation when compared with the soluble form, and can enhance antimicrobial activity when combined with other certain metal and silicate complexes.

In this work, antimicrobial activity was conferred to a nanocomposite consisting of carboxylated carbon nanotubes (CNTs-COOH)

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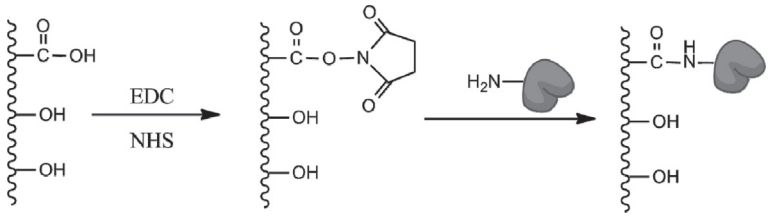

Fig. 1. Schematic representation of the CNTs-COOH/RC activation and enzyme immobilization.

or graphene oxide (GO) and regenerated cellulose (RC) by way of lysozyme immobilization through a lysozyme-amide-linked cellulose conjugate that was electrospun into nonwoven mats. Carboxyl groups on CNTs-COOH (or GO) were activated to react with the nucleophilic amino functionality at the side chains of amino acids in lysozyme [32]. The presence of carboxyl functional groups when combined with oxidized and regenerated cellulose provides an active site for chemical activation and functionalization necessary to attach proteins onto the cellulose surface [33]. A functionalization process is highly desired to immobilize lysozyme. Carboxyl carbon nanotubes (or GO) were utilized in the fabrication of electrospun cellulose nonwoven mats and supplemented with regenerated cellulose from recycled blue jeans. The purpose of this work was to demonstrate the feasibility of engineering a one-pot process for enzyme immobilization on in nanocomposites, and to further verify bioactivity of the resulting lysozyme-cellulose conjugate attached on the regenerated cellulose nonwoven mat.

2. Materials and experiments

2.1. Materials

Raw cellulose was prepared from recycled blue jeans. Carboxyl carbon nanotubes (10–20 nm diameter, 10–30 μm length, 95% purity) was purchased from Nanostructured & Amorphous Materials Inc. Ionic liquid solvent 1-butyl-3-methylimidazolium chloride ([BMIM]Cl) with purity > 95% and lysozyme (2000 U mg^{-1}) from chicken egg white were from Sigma-Aldrich, Inc. N-Hydroxysuccinimide (NHS) was from Thermo Inc., and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) was from TCI, Inc.

2.2. Formation of CNTs-COOH/RC and GO/RC nonwoven mats

A piece of blue jeans (5.0 g) was bleached with 50 mL sodium hypochlorite solution (30 wt%) for 3 h, and then rinsed with distilled water and dried at room temperature. The dried bleached jeans were ground with a domestic grinder to get recycled cellulose. Solid [BMIM]Cl was heated to 80 °C in a water bath to melt completely. In order to get a well-dispersed CNTs-COOH suspension, an amount of CNTs-COOH (8 and 10 wt% of cellulose respectively) was added in 30 mL melted [BMIM]Cl and ultrasonicated for 4 h. Then 0.9 g of recycled cellulose was dissolved in the suspension of CNTs-COOH. The mixing operation was run at 90 °C with vacuuming and a 40-rpm mechanical agitation. A CNTs-COOH suspended cellulose solution was obtained after heating and kneading for 1 h. The solution was immediately fed into a 20-mL metallic syringe for dry-wet electrospinning.

GO-suspended cellulose solution was prepared in the same method. GO solution was prepared from natural graphite by a modified Hummer's method [34]. An amount of 2.7 g of GO solution (1.0 wt%) was added in 30 mL melted [BMIM]Cl to form GO suspension after ultrasonicating for 1 h. A quantity of 0.9-g recycled cellulose was dissolved in the suspension, and then the mixing operation was heated and stirred at 90 °C with vacuuming and a 40-rpm mechanical agitation for 1 h.

The dry-wet electrospinning equipment in this study is a lab-scale apparatus made up of four parts: a syringe pump, a syringe with constant temperature cover, a high voltage source, and a water bath collector. In the electrospinning process, the syringe with a blunt metallic needle (24 G, 0.3 mm i. d.) was placed vertically above the collector. The distance between the tip of the needle and the surface of water bath was kept at 9 cm. Temperature of the heating cover was set up at 95 °C. The applied voltage was maintained at 10 KV. After the electrospinning, the collected fibers was immersed in deionized water for 48 h at room temperature to remove [BMIM]Cl, and dried at room temperature to get CNTs-COOH/regenerated cellulose nonwoven nanofibrous mats (CNTs-COOH/RC) or GO/RC.

2.3. Immobilization of lysozyme on CNTs-COOH/RC

Lysozyme (Lys) was covalently attached onto CNTs-COOH/RC and GO/RC based on the EDC/NHS activation procedure described in the literature [35]. The synthetic steps are shown in Fig. 1. The produced nonwoven nanofibrous mat was rinsed using phosphate buffer solution (PBS, 50 mM, pH = 7.0). The rinsed mat was immersed in PBS (50 mM, pH = 7.4) containing 500 mM NHS, followed by an addition of 20 mM EDC. The system was stirred at 200 rpm for 4 h to initiate the coupling of NHS to the carboxyl groups on CNTS-COOH or GO. The activated mat was taken out from the system and rinsed thoroughly with PBS to remove excess EDC and NHS. Then the mat was immersed in a lysozyme solution (0.05 mg·mL^{-1} in PBS, pH = 7.0). Enzyme immobilization was conducted at room temperature for 4 h to get Lys@CNTs-COOH/RC and Lys@GO/RC.

2.4. Characterization of Lys@CNTs-COOH/RC

2.4.1. Scanning electron microscopy (SEM) analysis

The morphology of CNTs-COOH/RC and Lys@CNTs-COOH/RC was observed by SEM (Zeiss Supra40, Carl Zeiss Inc., Germany) at 5.00 KV. Prior to the observation, the mats were coated with gold/platinum using a sputtering coater.

2.4.2. Fourier transform-infrared (FT-IR) spectra

FT-IR spectra of all samples were obtained using a Thermo Mattson Infinity Gold FT-IR. A total of 16 scans were carried out on wavenumbers from 4000 to 400 cm^{-1} in transmittance mode.

2.4.3. Thermal-gravimetric (TG) analysis

TG analysis and derivative thermal gravity (DTG) were conducted under air flow with elevated heating from room temperature to 900 °C at a heating rate of 5 °C min^{-1} (TGA 50, Shimadzu Co, Japan).

2.5. Tensile properties

The tensile properties of RC nanofibrous mats and CNTs-COOH/RC were studied using a tensile tester (5966 Dual Testing Systems, Instron)