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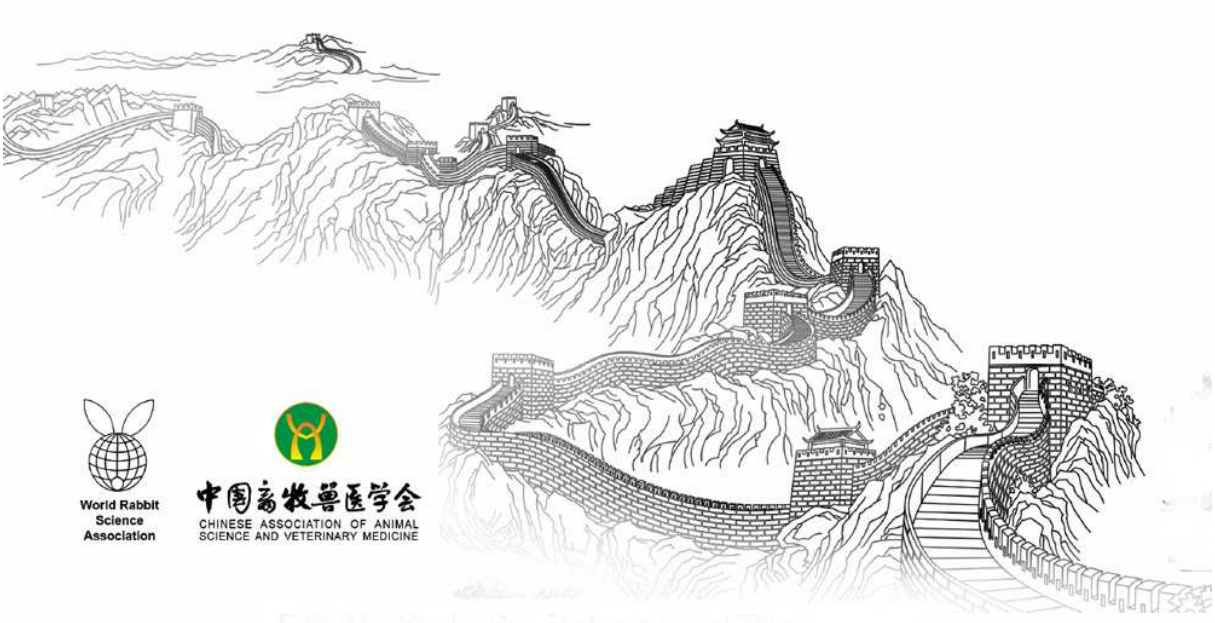

CHARACTERIZATION OF THE MICRO STRUCTURE AND ANTIBACTERIAL PROPERTIES OF THE HAIR FIBER MODIFIED BY PEGYLATED CHITOSAN

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ABSTRACT

In this work, poly-ethylene glycol (PEG) grafted chitosan (PEG-g-CS) was synthesized by conjugating PEG to the chitosan (CS) backbone. Such PEGylated chitosan copolymer was characterized by ^{1}H Nuclear Magnetic Resonance (NMR). Chitosan could be used as water borne coating for hair fiber. To anchorage the chitosan derivative to the hair fiber, UV was conducted. The micro structure of coating on hair fiber was studied by Scanning Electron Microscope (SEM). Furthermore, the antimicrobial activity of the hair fiber modified by PEGylated chitosan was investigated by measuring its minimum inhibitory concentration (MIC) and the shake-flask culture method against two strains. The results showed that PEGylation modified chitosan exhibited better antibacterial property than that of chitosan.

Key words: PEGylation; chitosan; hair fiber; micro structure; synergistic antimicrobial effect

INTRODUCTION

Now days, consumers prefer to purchase more hygiene hair fiber product (Fernandes *et al.,* 2013). However, the growth and large propagation of these microbes on the hair fiber surface will bring potential health problems to consumers (Orlita, 2004). To address it, the antibacterial hair fiber was prepared by using the natural antimicrobial agents. As we all know, chitosan is being currently proposed for a wide range of applications due to antimicrobial activity and non-toxicity (Liu *et al.,* 2015; Liu *et al.*, 2004; Marziyeh *et al.*, 2013; Lee *et al.*, 2012). However, chitosan is only soluble in aqueous acidic solutions below pH 6.5. To combat these adversities and have better antibacterial effect, we have been made to select PEGylation to successfully modify chitosan in this work.

MATERIALS AND METHODS

Materials

Chitosan (Mw = 8 KDa, DD≥ 95.0%) was purchased from Nantong Xingcheng Biological Industrial Co., Ltd. (Jiangsu, China). Methoxy-polyethylene glycols (Mw = 1 KDa) and glutaraldehyde were purchased from Sinopham Chemical Reagent Co., Ltd. All of the other reagents used in this paper were obtained from Sigma-Aldrich. The hair fiber from the rex rabbit were prepared in our lab.

Synthesis of PEGylated Chitosan

The synthesis of PEGylated chitosan was prepared by grafting PEG-NO_2 onto chitosan. Briefly, 10 g of CS was dissolved in 100 mL 1 wt % of acetic acid aqueous solution to obtain homogeneous solution. Then, 3 g of the PEG-NO_2 was added to the CS solution and stirred for 48 h. After that, the reaction

product was dialyzed to remove the p-nitrophenol dropped or unreacted PEG-NO_2. Finally, the PEG-g-CS copolymer was obtained by freeze drying.

Coating of hair fiber surface with PEG-g-CS
Briefly, 2 g of the hair fiber irradiated for 30 min by UV was added to the PEG-g-CS solution before 0.1 wt % of the antibacterial agent was prepared. The hair fiber was then irradiated again. After that, the treated hair fiber was washed by deionized water and then dried to obtain the antibacterial hair fiber.

Characterization
^{1}H NMR spectra of the polymers were recorded in D_2O using a Bruker 400 NMR spectrometer and the polymer's concentration was 20 mg mL^{-1}. Micro structure of the hair fiber was evaluated through SEM with Phenom G2 pure.

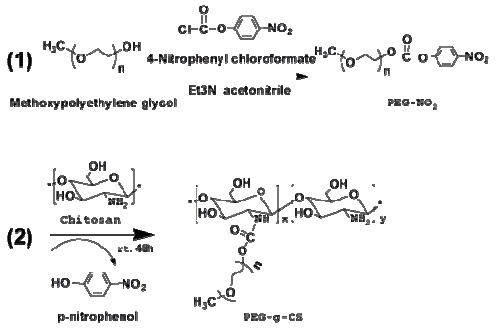
Antimicrobial activity assays
Antimicrobial test of polymers and its coating were conducted quantitatively by the MIC test and shake-flask culture method, respectively, with *E. coli* and *S. aureus* as model bacteria.

Minimum inhibitory concentration test
In the assay of MIC, the different copolymers with the same concentration (0.2 g mL^{-1}) were used. 1 mL of these solution samples were diluted to different concentrations. Then, the above solutions were added to 5 mL of LB medium with tested bacterial concentrations of 10^7 cfu mL^{-1}. The isometric sterile water was used as the control. The tubes were incubated at 37 °C with shaking at 200 rpm for 18 h. After incubation, a desired volume of the solution was to determine colonies of viable cells. Antibacterial activity was defined as the percentage of microbe reduction (Lee et al. 2012). The percentage of colony number reduction was then determined according to the following equation: $\text{Re}\,duction\ (\%) = \frac{(B-A)}{B} \times 100$
where A is colony counting for the flask containing the sample and B is the colony counting for the flask containing the sterile water as control. According to the result, the MIC was defined as the lowest samples concentration resulting in the percentage of microbe reduction ≥ 90%.

Shake-flask culture testing
In the shake-flask culture method, the treated hair fiber was placed in test tubes containing 10 mL 1×10^5 CFU mL^{-1} of two strains in sterile water. These tubes were shaken at 200 rpm in incubators for 37 °C. At a predetermined time, cells were pipetted out from the tubes. 0.2 mL was then plated onto the triplicate solid agar using the spread plate method. After incubating for 24 h, the number of viable bacteria was counted and the results.

RESULTS AND DISCUSSION

Synthesis of PEG-g-CS copolymer
The hydrophilic PEG was firstly activated to generate PEG-NO_2 which can react with the amino groups of CS in Scheme 1. In Figure 1a, the signals located at δ 7.4 (g, f) and δ 8.3 (h, i) ppm belonged to the benzene ring. While δ 3.1 (a) and δ 3.6 (b, c, d) ppm were attributed to methyl and methylene group, respectively. Based on the results, PEG-NO_2 was synthesized successfully. In step two PEG-g-CS copolymers were further synthesized by reacting between PEG-NO_2 and CS. Compared to the ^{1}H NMR spectrum of chitosan, the peaks at δ 3.1 (a) and δ 3.6 (b, c, d) ppm of PEG-g-CS were assigned to the protons of PEG chains (Figure 1b).



Scheme 1: Synthetic route of PEGylated Chitosan

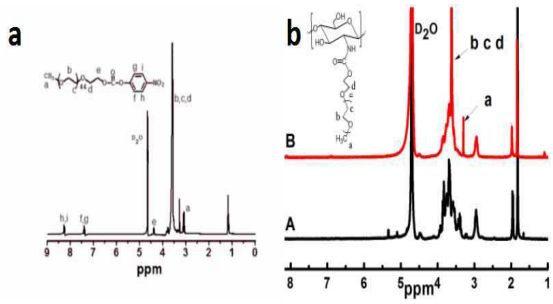


Figure 1: ^{1}H NMR spectra of (a) PEG-NO_2 and (b) CS and PEG-g-CS in D_2O solvent

Characterization of micro structure of the treated hair fiber

SEM micrographs (Fig. 2a and 2b) demonstrate the depositions of PEG-g-$CS_{8\%}$ on the hair fiber. The SEM image in Fig. 3a demonstrates the structure of the leather before coating. After treating, the deposition of PEG-g-$CS_{8\%}$ on the hair fiber was shown in Fig. 2b.

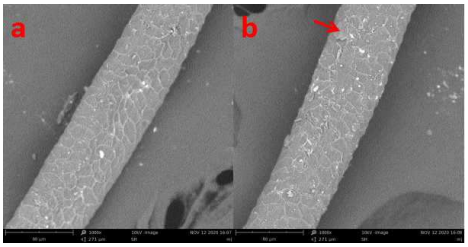


Figure 2: The SEM micrograph of treated Hair fiber embedded with (a) control Fiber and (b) PEG-g-$CS_{8\%}$.

Antimicrobial property of PEG-g-CS copolymer and its coating for hair fiber

Two bacterial strains[6] were used to evaluate the bactericidal activities of hair fiber loaded with various materials using minimum inhibitory concentration and Shake-flask culture testing. MIC of PEG-g-CS copolymers was firstly investigated by using *E. coli* and *S. aureus* as bacterial models, (Hu *et al.*, 2005) and CS was used as the control. Therefore, the PEG-g-CS copolymer and CS solution (0.2 g mL^{-1}) were diluted to different concentrations. It is obvious that MIC value of PEG-g-CS copolymer was much smaller than that of CS, indicating the higher antimicrobial activity of PEGylated chitosan. The MIC values were detected at 25 mg mL^{-1}, 3.12 mg mL^{-1} and 0.78 mg mL^{-1} for CS, PEG-g-$CS_{4\%}$ and PEG-g-$CS_{8\%}$, respectively. Therefore, the excellent antimicrobial property of PEGylated chitosan was mainly due to its PEG content.

Shake-flask culture method

In this method, the hair fiber was incubated with two strains suspension for 5 h. The result was then used for plating and colony counting. The number of viable cells decreased about 3% after 5 h in the control because of the natural death in figure 3. There was obvious reducing of *E. coli* concentration showing the absence of PEG constituent between CS and PEG-g-CS. The result was the same to *S. aureus*. PEG-g-$CS_{8\%}$ had higher antibacterial owing to the PEG constituent than PEG-g-$CS_{4\%}$

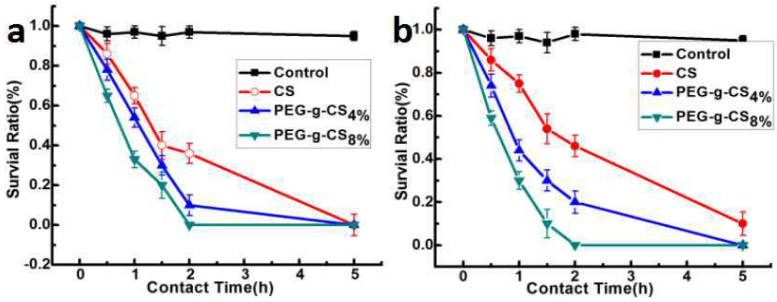


Figure 3: Changes in the viable (a) *E. coli* and (b) *S. aureus* cells after exposure to CS, PEG-g-$CS_{4\%}$ and PEG-g-$CS_{8\%}$ modified hair fiber respectively. The untreated sample is as control.

CONCLUSIONS

In conclusion, CS derivatives were successfully synthesized and well-characterized by using ^{1}H NMR spectroscopies. These composites showed good antibacterial activity in both shake-flask culture method and MIC test, and the antibacterial activity of the hair fiber modified by PEG-g-$CS_{8\%}$ was found to exhibit higher antibacterial activities than pure CS and PEG-g-$CS_{4\%}$ were observed in both cases. These results, therefore, demonstrate that the introduction of PEGylation with the hydrophilic property will enhance the antibacterial properties of CS. The chitosan derivative has great potential for use in hair fiber products.

ACKNOWLEDGEMENTS

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