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Synthesis of PEGylated chitosan copolymers as efficiently antimicrobial coatings for leather

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ABSTRACT: In this work, poly(ethylene glcycol)-grafted chitosan (PEG-*g*-CS) was synthesized by conjugating PEG to the chitosan (CS) backbone. Such PEGylated CS copolymer was further characterized by FTIR and ^{1}H NMR, and the results demonstrated the successful synthesis. After PEGylation, the water solubility of CS was significantly improved due to the hydrophilicity of the PEG polymer. Therefore, this PEGylated CS was prepared as water borne coating for leather surface. The morphology and hydrophilicity of this coating on leather was studied by SEM and water contact angle measurement. Furthermore, the antimicrobial activity of PEGylated CS coating was investigated by measuring its minimum inhibitory concentration and the inhibition zone of coated leather against Gram-negative *Escherichia coli* and Gram-positive *Staphylococcus aureus*, respectively. Compared to CS coating, such PEG-*g*-CS coating exhibited better antimicrobial property, which indicated the synergetic effect of the antimicrobial property of CS and the antiadhesive property of PEG. Thus, this PEGylated CS copolymer can be used as efficiently antimicrobial coating for leather product. © 2016 The Authors Journal of Applied Polymer Science Published by Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2016**, *133*, 43465.

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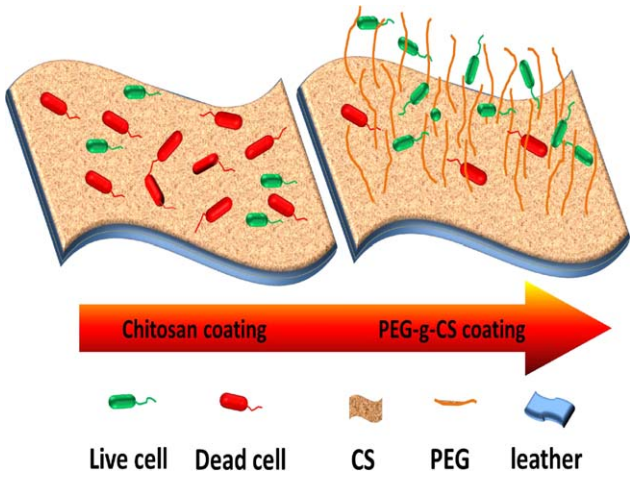
INTRODUCTION

Nowadays, consumers prefer to purchase more hygiene leather product. However, leather with collagen network actually acts as an excellent harbor for microbes, and it can provide ideal conditions such as moisture, temperature, oxygen, and nutrient required for the rapid growth of bacteria and fungi.[1] More seriously, the growth and large propagation of these microbes on the leather surface will bring potential health problems to consumers.[2] For example, in footwear, the surface colonization and microorganism' growth not only cause material deterioration and generate unpleasant smell, but also bring risks of infections.[3] Certain microorganisms are responsible for causing skin infections that can be quite lengthy, difficult to eradicate, and damaging.[4] Especially for susceptible individuals, such as diabetic patients are more susceptible to these microorganisms and the infection will result in foot ulceration and amputation.[4,5] Therefore, the inhibition of microorganism's growth on the leather surface has been an important issue.

To overcome these problems, much effort has been concentrated on fabricating leather products with antimicrobial properties.[6] Unfortunately, many of the chemicals and strategies used to impart antimicrobial property into leather are not eco-friendly, which are usually toxic to humans and lead antibiotic-resistant bacteria.[7] For instance, in leather industry, chemical biocides are often introduced to the leather making process to reduce the bacteria.[8] However, they mainly play the role in preserving hides during processing rather than to confer antimicrobial properties to the final products.[9] Moreover, some of these biocides have been restricted due to human health and environmental issues.[7] Thus, the development of eco-labeled and effectively antimicrobial coatings for final leather products become an ideal strategy, which will benefit a lot to both industry and consumers.

To construct antimicrobial coating for leather, the economical, nontoxic and effectively antimicrobial materials is the primary consideration. Due to the eco-friendly and nontoxic to humans, silver nanoparticles (Ag NPs) have been attracting high interest and used as coating materials in the industrial, medical and environmental fields.[10,11] Recently, Byung-Taek Oh's group used green-synthesized Ag NPs to coat on the leather surface for getting



Scheme 1. PEG-*g*-CS coating exhibited more efficiently antimicrobial property than that of chitosan coating, due to the anti-adhesive PEG chains. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

rid of bad odors and bacterial infections.[12] Chitosan (CS), the linear and partly acetylated (1-4)-2-amino-2-deoxy-β-D-glucan isolated from marine chitin, is being currently proposed for a wide range of applications due to antimicrobial activity, biocompatibility, nontoxicity, and biodegradability.[13–17] Barreiro et al. developed an antimicrobial leather coating based on CS, taking advantages of CS intrinsic antimicrobial activity and film-forming capacity.[1] Such kind of coated leather with antimicrobial properties was supposed to be used in footwear components.

Despite the advantages of eco-friendly and antimicrobial property of above leather coatings, the antimicrobial efficiency is still worth investigating. The antimicrobial mechanisms of Ag NPs or CS coatings are mainly based on destroying the microorganism cell membrane, finally leading to cell death.[11,14] After a certain time, the dead microorganism on the surface will affect or shield the antimicrobial activity of these coatings, resulting in lower antimicrobial efficiency. To address this issue, we designed and synthesized a PEGylated chitosan copolymer (PEG-*g*-CS) to fabricate high antimicrobial efficiency coating with nonfouling properties for leather (Scheme 1). After PEGylation, the water solubility of CS was significantly improved, which overcome its limitations of utilization in acidic solutions. What is more, the PEGylated CS can effectively prevent the adhesion of microorganism on the coating, due to the antifouling properties of PEG.[18–21] Hence, the combination of the antimicrobial CS and antiadhesive PEG make such PEGylated CS copolymer can be used as efficiently antimicrobial coating for leather product.

EXPERIMENTAL

Materials

CS ($M_w = 8$ KDa, DD $\geq 95.0\%$) was purchased from Nantong Xingcheng Biological Industrial (Jiangsu, China). Methoxypolyethylene glycols (PEG, $M_w = 1$ KDa) was purchased from Sinopham Chemical Reagent. Triethylamine, 4-nitrophenyl chloroformate, 2,4,6-trinitro-benzene sulfonic(TNBS), and acetonitrile were obtained from Sigma-Aldrich. Leather samples were prepared followed the wet blue leather tanning process in our laboratory.

Synthesis of PEGylated Chitosan

To obtain the PEGylated CS, PEG-nitrophenyl carbonate (PEG-NO_2) was first synthesized by reacting PEG and 4-nitrophenyl chloroformate according the literature with the yield of around 86%.[22] Then, the synthesis of PEGylated CS was prepared by grafting PEG-NO_2 onto CS. Briefly, 10 g of CS was dissolved in 100 mL of 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 placed in dialysis solution bags (MWCO 3500), and dialyzed in distilled water for 48 h by changing the water every 10 h to remove the *p*-nitrophenol dropped or unreacted PEG-NO_2. Finally, the PEG-*g*-CS copolymer was obtained by freeze drying for 3 days with the yield of about 54%.

Determination of the Substitution of PEG-*g*-CS

The amino substitution degree (SD) of PEG-*g*-CS defined as the number of PEG group per 100 amino groups of CS, was determined by TNBS method.[23] To determination of the substitution of PEG-*g*-CS, a calibration curve was first made. 0, 50, 100, 200, 500, 800, and 1000 μL CS solutions (1.0 mg mL^{-1}) were transferred to 10 mL centrifuge tubes and diluted with water to 3 mL, respectively. Every sample was incubated with 2 mL of 4% $NaHCO_3$ and 2 mL of 0.1% TNBS under 37 °C for 2 h. 2 mL of 2 mg mL^{-1} HCl was then added. The ultra-violet absorbance of samples at 344 nm was measured by UV spectroscopy (UV-2502, Shimadzu Corporation). The calibration curve could be obtained by these samples with different CS concentrations.

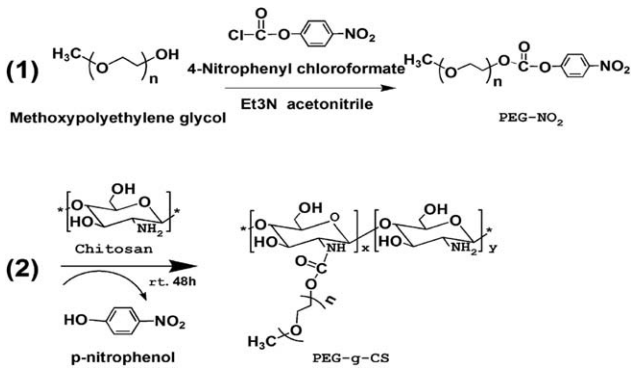
In this work, two kinds of PEG-*g*-CSs, such as PEG-*g*-$CS_{4\%}$ and PEG-*g*-$CS_{8\%}$, were synthesized. Thus, for determining the SD of PEG-*g*-$CS_{4\%}$ and PEG-*g*-$CS_{8\%}$, 3 mL PEG-*g*-CS solution (0.1 mg mL^{-1}) was incubated with 2 mL of 4% $NaHCO_3$ and 2 mL of 0.1% TNBS under 37 °C for 2 h. Then, 2 mL of 2 mg mL^{-1} HCl was added. The absorbance of samples at 344 nm was measured by UV spectroscopy. The SD of PEG-*g*-CS was calculated using the calibration curve.

Preparation of PEG-*g*-CS Coating onto Leather Surface

Briefly, leather was immersed in 40 mg mL^{-1} solutions of PEG-*g*-$CS_{4\%}$, PEG-*g*-$CS_{8\%}$, and CS, respectively. After stirring for 2 h, the leather was dried under room temperature. Then circular leather sample with diameter of 13 mm was prepared for SEM analysis, water contact angle analysis, and zone of inhibition (ZOI) test. Each experiment was performed in triplicates and the final values were presented as the mean ± standard deviation.

Characterization

FTIR spectra were recorded on a Nicolet Nexus 670 FTIR spectrophotometer using KBr pellets and were scanned against a blank KBr pellet background at wave numbers in the range of 4000–500 cm^{-1} with a resolution of 4.0 cm^{-1} at 25 °C. For each sample 32 scans were carried out. ^1H NMR spectra of the polymers were recorded in D_2O using a Bruker 400 NMR spectrometer (Bruker) at 25 °C, and the concentration of the polymer was 20 mg mL^{-1}. Morphology modification of the leather sample surface due to sample coating was evaluated through scanning electron microscopy (SEM; Phenom, China). Water contact angle was measured to character the hydrophilic of each sample by using OCA20 (Dataphysics, Germany). For each sample, water contact angle was measured at



Scheme 2. Synthetic route of PEGylated Chitosan.

six places within 15 s after the secondary distilled water droplet contacted the surface and the average contact angle was reported.

Antimicrobial Activity Assays

Antimicrobial test of polymers and its coating were conducted quantitatively and qualitatively by the minimum inhibitory concentration (MIC) test and ZOI test, respectively, with *Escherichia coli* (ATCC 51813) and *Staphylococcus aureus* (ATCC 27661) as model bacteria.

Minimum Inhibitory Concentration Test

In detailed, *E. coli* and *S. aureus* were cultivated in Luria Bertani (LB) medium (containing 10 g L^{-1} peptone, 5 g L^{-1} yeast extract, and 10 g L^{-1} sodium chloride). The solid medium was solidified by 20 g L^{-1} agar. The pH was adjusted to pH 7.0–7.2 with 1*M* NaOH before autoclaving. Autoclaved Luria Bertani (LB) medium that had been allowed to cool to approximately 40 °C was added to the petri dish for antimicrobial activity assays. In the assay of MIC testing, the solutions of CS, synthesized PEG-*g*-$CS_{4\%}$ and PEG-*g*-$CS_{8\%}$ with the same concentration (0.2 g mL^{-1}) were used. 1 mL of these solution samples were diluted to different concentrations by using sterile water, and the diluted ratios were set as follows from 1/2, 1/4, 1/8, to 1/512. Then, the above solutions with different diluted ratios were added to 5 mL of Luria Bertani (LB) medium with tested

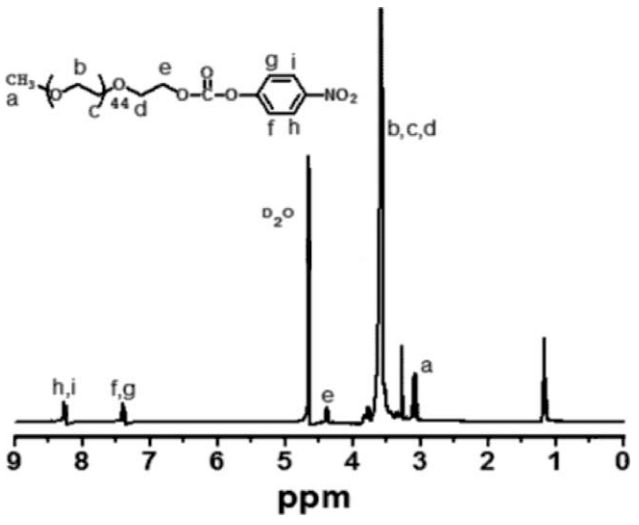


Figure 1. ^{1}H NMR spectra of PEG-NO_2 in D_2O solvent.

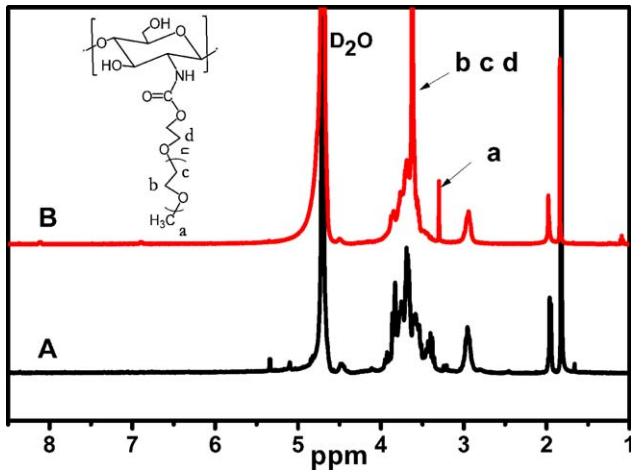


Figure 2. ^{1}H NMR of (A) CS and (B) PEG-*g*-CS in D_2O solvent. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

bacterial concentrations of 10^7 cfu mL^{-1} (cfu = colony-forming units). For the blank samples, the isometric sterile water was added to the control tube. The tubes were incubated at 37 °C with shaking at 200 rpm for 18 h in a constant-temperature incubator. After incubation, a desired volume of the solution was to determine colonies of viable cells by standard plate count techniques. Antibacterial activity was defined as the percentage of microbe reduction.[24] The percentage of colony number reduction was then determined according to the following equation:

$$\text{Reduction}\,(\%) = \frac{(B-A)}{B} \times 100$$

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 $\geq$ 90%.

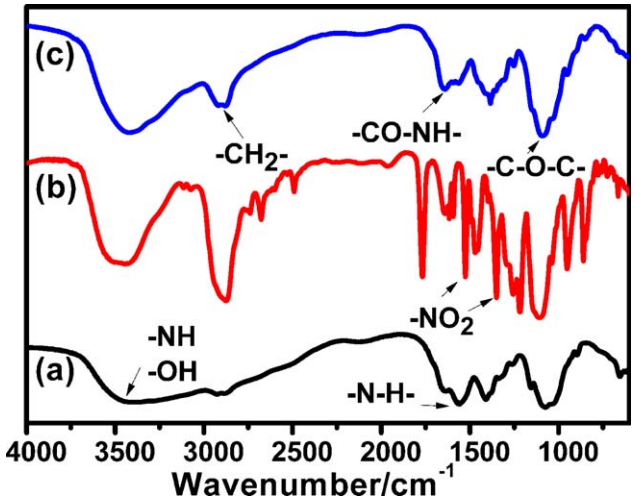


Figure 3. FTIR spectra of (a) CS, (b) PEG-NO_2, and (c) PEG-*g*-CS. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

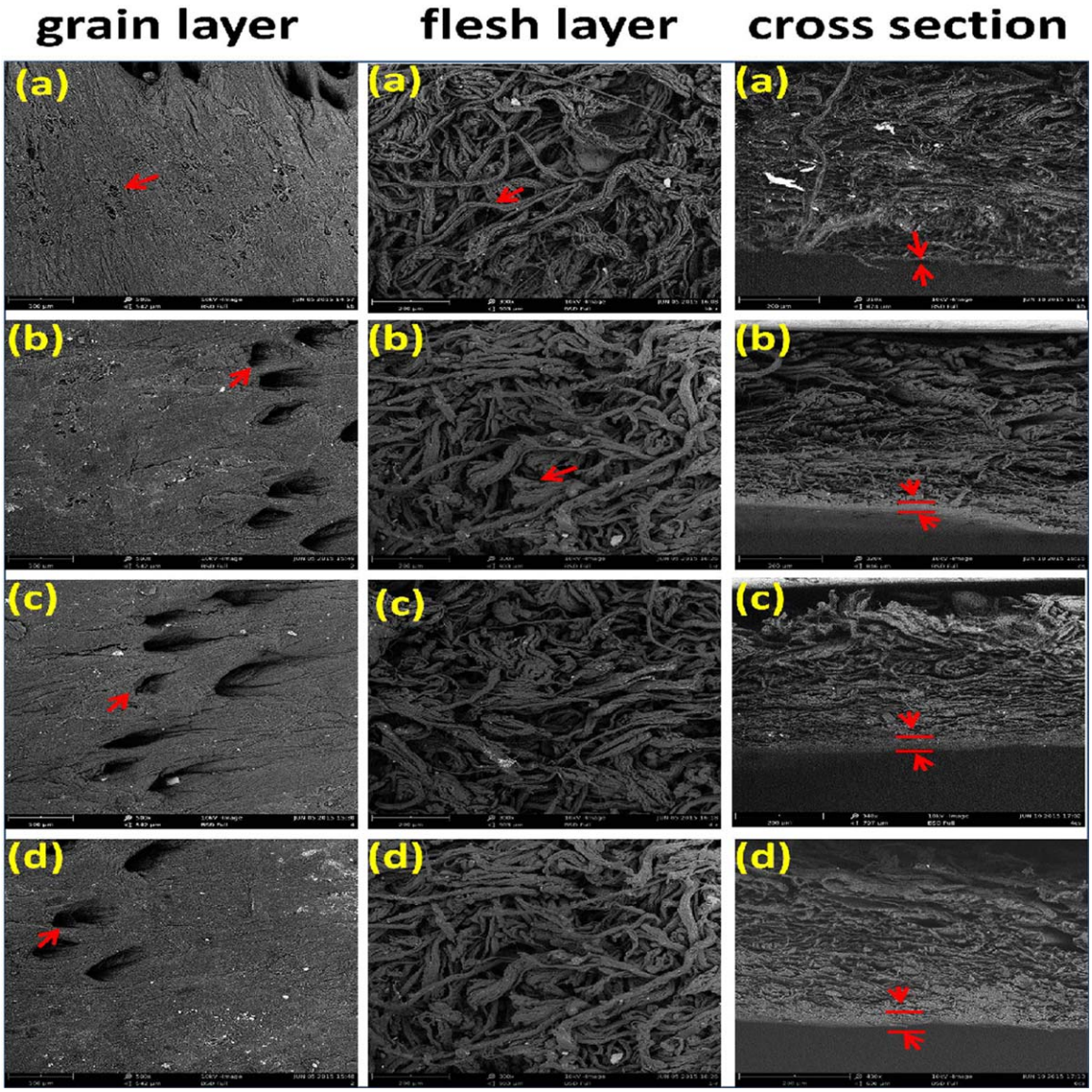


Figure 4. SEM images of leather coated with (a) water as control, (b) CS, (c) PEG-*g*-$CS_{4\%}$ and (d) PEG-*g*-$CS_{8\%}$. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Zone of Inhibition Test

Leather sample with diameter of 13 mm were placed on agar plate coated 20 μL suspension of *E. coli* and *S. aureus* and incubated at 37 °C for 18 h. Then, the plates were examined for possible clear zone formation after incubation. The presence of clear zone around the samples was recorded as an inhibition against the bacterial strains. At last, the diameter of such ZOI was measured using a meter ruler and the mean value for each organism was recorded and expressed in millimeters.

RESULTS AND DISCUSSION

Synthesis of PEG-*g*-CS Copolymer

The detailed synthesis procedure of PEGylated CS copolymer is shown in Scheme 2. Briefly, the hydrophilic PEG was first activated to generate PEG-NO_2 which can react with the amino groups of CS, and its chemical structure was evidenced by ^{1}H NMR. As shown in Figure 1, 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.

As shown in step two of scheme 2, PEG-*g*-CS copolymers were further synthesized by reacting between PEG-NO_2 and CS. During the process of reaction, the *p*-nitrophenol dropped from PEG and amide bonds formed between PEG and CS. Therefore, PEG can be grafted onto the CS backbone. Compared to the ^{1}H NMR spectrum of CS, 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 2). Moreover, the absence of the characteristic peaks of nitrobenzene group at δ 7.1 and δ 8.3 ppm also demonstrated the successful synthesis of the PEG-*g*-CS copolymers. FTIR spectroscopy was

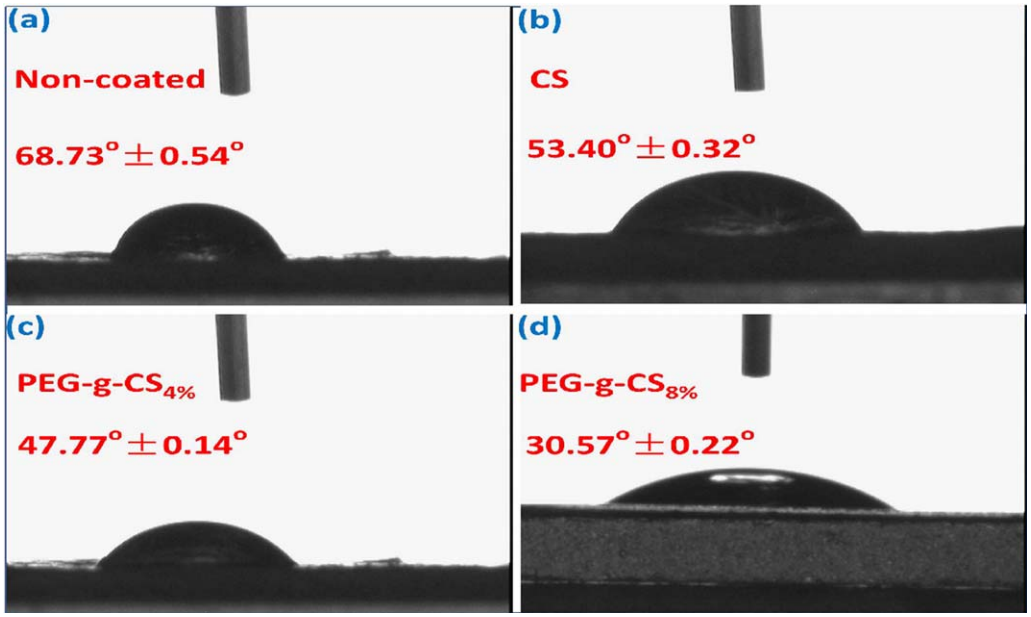


Figure 5. Water contact angle of leather coated with (a) nothing as control; (b) CS; (c) PEG-*g*-$CS_{4\%}$ and (d) PEG-*g*-$CS_{8\%}$. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

further employed to prove the successful synthesis of the PEGylated CS. AS shown in Figure 3, compared to CS and PEG-NO_2, the FTIR result of PEG-*g*-CS provided clear evidence that the peaks at 2927 cm^{-1} and 1027 cm^{-1} corresponds to the antisymmetric stretching of —CH_2— and —C—O—C— groups on the PEG chains, respectively. While the peak at around 1649 cm^{-1} due to the -CO-NH- groups resulting from the reaction of PEG-NO_2 and CS.[16] In this work, two kinds of PEG-*g*-CS copolymer with different amino-SDs were synthesized. The amino-SDs of PEG-*g*-CS copolymer were calculated to be 2.59 ± 0.79 and 7.28 ± 2.17% for PEG-*g*-$CS_{4\%}$ and PEG-*g*-$CS_{8\%}$, respectively.

Preparation and Characterization of PEG-*g*-CS Coating on the Leather Surface

Surface coatings on the leather surface were prepared by soaking leather samples into the solutions of CS, PEG-*g*-$CS_{4\%}$, PEG-*g*-$CS_{8\%}$, and water was used as the blank control. The concentration of the polymer was set at 40 mg mL^{-1}. After drying, the coating layer could be formed on the leather surface, and the morphologies of these coatings were evaluated via SEM. Figure 4 showed the morphologies of water-coated leather and polymer coated leather. Compared with Figure 4(a), the grain layer of CS or PEG-*g*-CS-coated leather showed a more uniform and smooth surface with low roughness. In addition, the pores appeared on the original leather surface caused by damage [red arrow, Figure 4(a)] were filled significantly after the polymer coating [Figure 4(b,c,d]. For the flesh layer, compare to in Figure 4a of the uncoated leather images, Figure 4(b, c,d) revealed the CS and PEGylated CS coatings obviously adhering to collagen fibbers. Moreover, it was also observed that the coating thicknesses in cross section was thicker than that of the blank control, which were about 21.1 ± 2.2, 23. 6± 4.5, and 26.4 ± 4.4 μm for CS, PEG-*g*-$CS_{4\%}$, and PEG-*g*-$CS_{8\%}$ coatings, respectively. The above results revealed that the leather had been successfully coated by different materials.

It is well known that PEG polymer can tightly bind water molecules via the hydrogen bonding, thus resulting into its excellent hydrophilixity.[25] After PEGylation, the water solubility of CS could be significantly improved due to the hydrophilic PEG polymer. Therefore, the PEGylated CS can be used as water borne coating for leather surface. The hydrophilicity of PEG-*g*-

Table I. MIC Results of CS and PEG-*g*-CS against *E. coli* and *S. aureus*

		Dilution ratio							
Samples	Bacteria	1/2	1/4	1/8	1/32	1/64	1/128	1/256	1/512
CS	*E. coli*	−	−	−	+				
	S. aureus	−	−	−	+				
PEG-g-$CS_{4\%}$	*E. coli*	−	−	−	−	−	+		
	S. aureus	−	−	−	−	−	+		
PEG-g-$CS_{8\%}$	*E. coli*	−	−	−	−	−	−	−	+
	S. aureus	−	−	−	−	−	−	−	+

"−" is the value of the percentage of microbe reduction ≥90%; "+" is the value of the percentage of microbe reduction <90%.

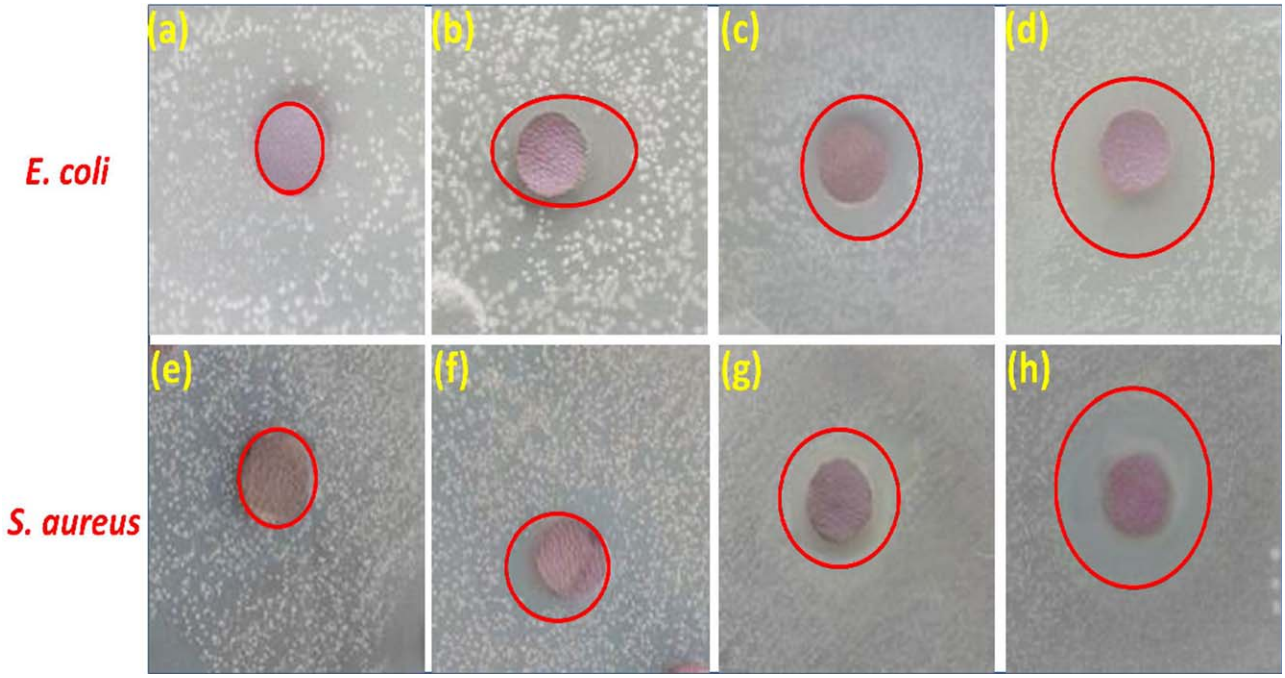


Figure 6. Zone of inhibition observed with two bacterial culture plates containing leather loaded with (a,e) nothing as control, (b,f) CS, (c,g) PEG-*g*-$CS_{4\%}$, and (d,h) PEG-*g*-$CS_{8\%}$. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

CS coating was studied by water contact angle (WCA), and CS coating was used as blank control. As shown in Figure 5, after CS coating, the WCA of the leather surface decreased from 68.73° to 53.40°. While the PEG-*g*-CS coatings dramatically showed smaller WCAs which were 47.77° and 30.57° for PEG-*g*-$CS_{4\%}$ and PEG-*g*-$CS_{8\%}$, due to their higher hydration of the coating surfaces. Actually, effective hydration of the coating is critical for non-fouling properties, avoiding the adhesion of microorganisms.[26,27] Therefore, such PEG-*g*-CS coating was supposed to be effective in preventing microorganisms' adsorption.

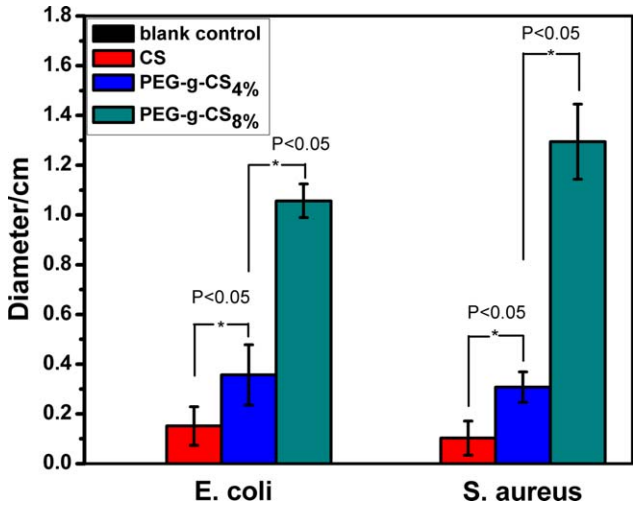


Figure 7. Histogram is showed about the degree of the diameter for each sample. Error bars represent mean ± SD ($n \geq 3$). *P was determined by Student's t-test. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Antimicrobial Property of PEG-*g*-CS Copolymer and Its Coating for Leather

To evaluate the antimicrobial property of the PEG-*g*-CS coating for leather product, the MIC of PEG-*g*-CS copolymers was first investigated by using Gram-negative *E. coli* and Gram-positive *S. aureus* as bacterial models,[28] and CS was used as the control. According to the literature,[24] the MIC value was usually defined as the lowest material concentration resulting in the percentage of microbe reduction ≥90%. Therefore, in this antimicrobial activity test, the PEG-*g*-CS copolymer and CS solution (0.2 g mL^{-1}) were diluted to different concentrations. The MIC value will be the diluted concentration which just gives the percentage of reduction >90%. As shown in Table I, 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 CS. The MIC values were detected at 25, 3.12, and 0.78 mg mL^{-1} for CS, PEG-*g*-$CS_{4\%}$, and PEG-*g*-$CS_{8\%}$, respectively. The excellent antimicrobial property of PEGylated CS was mainly due to its hydrophilicity. After PEGylation, the water solubility of PEG-*g*-CS was improved and the copolymer chains could be more stretch than that of CS in the bacterial solution. Therefore, the more amino groups of PEG-*g*-CS could contact with bacteria, leading the death of them.

For the antimicrobial property of PEG-*g*-CS coating on the leather surface, the ZOI test was further performed. Leather samples coated with nothing, CS, PEG-*g*-$CS_{4\%}$, and PEG-*g*-$CS_{8\%}$ were incubated with bacterial strains together for 18 h at 37 °C, showed in Figure 6. The average sizes of the inhibition zone of these leather samples were summarized in Figure 7. The results demonstrated that PEG-*g*-CS coating exhibited significant advantages in antibacterial property, which consistent with

the MIC results. Furthermore, it worth noting that PEG-*g*-CS with higher PEG grafting ratio (PEG-*g*-$CS_{8\%}$) revealed much better antimicrobial activity than that of PEG-*g*-$CS_{4\%}$ coating. This can be attributed to the synergistic antimicrobial activities of PEG-*g*-$CS_{8\%}$ coating, in which more PEG component played the role in preventing the adhesion of bacteria on the coating and CS sustainably inhibited the growth of the live cell. Thus, the combination of the antimicrobial CS and antiadhesive PEG make such PEGylated CS copolymer can be used as efficiently antimicrobial coating for leather product.

CONCLUSIONS

In this work, PEG-*g*-CS copolymers were successfully synthesized by grafting hydrophilic PEG onto the backbone of CS. After PEGylation, the water solubility and antimicrobial activity of such CS derivatives were significantly improved. As antimicrobial coatings for leather product, PEGylated CS exhibited more efficiently antibacterial activity than that of single CS coating, due to the synergistic antimicrobial property. In this PEG-*g*-CS coating, PEG component can play the role in preventing the adhesion of bacteria on the coating surface while CS will sustainably inhibited the growth of the live cell. Therefore, these eco-friendly and effectively antimicrobial coatings are promising for final leather products, which will benefit a lot to both industry and consumers.

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REFERENCES

1. Fernandes, I. P.; Amaral, J. S.; Pinto, V.; Ferreira, M. J.; Barreiro, M. F. *Carbohydr. Polym.* **2013**, *98*, 1121.
2. Orlita, A. *Int. Biodeter. Biodegrad.* **2004**, *53*, 157.
3. Shahid-ul-Islam.; Shahid, M.; Mohammad, F. *Ind. Eng. Chem. Res.* **2013**, *52*, 5245.
4. Magdalena, M.; Natalia, S. N.; Francisca, C. G.; César, A. A. O. B. *J. Dispersion Sci. Technol.* **2011**, *32*, 1722.
5. Kanlayavattanakul, M.; Lourith, N. *Int. J. Cosmet. Sci.* **2011**, *33*, 298.
6. Ayca, A.; Kaplan, S. *Fibers Polym.* **2013**, *14*, 316.
7. Sirvaityte, J.; Siugzdaite, J.; Valeika, V. *Rev. Chim. Bucharest* **2011**, *62*, 884.
8. Širvaitytėa, J.; Šiugždaitėb, J.; Valeikac, V.; Dambrauskiene, E. *Est. Acad. Sci.* **2012**, *61*, 220.
9. Stockman, G.; Didato, D. T.; Hurlow, E. *J. Am. Leather Chem. Assoc.* **2007**, *102*, 62.
10. Lkhagvajav, N.; Koizhaiganova, M.; Yasa, I.; Çelik, E.; Sari, Ö. *Braz. J. Microbiol.* **2015**, *46*, 41.
11. De, Simone, S.; Lombardi, F. A.; Paladini, F.; Starace, G.; Sannino, A.; Pollini, M. *J. Appl. Polym. Sci.* **2015**, *132*, 41623.
12. Velmurugan, P.; Cho, M.; Lee, S. M.; Park, J. H.; Bae, S.; Oh, B. T. *Carbohydr. Polym.* **2014**, *106*, 319.
13. Liu, G. Y.; Luo, Q. Q.; Wang, H. B.; Zhuang, W. H.; Wang, Y. B. *RSC Adv.* **2015**, *55*, 70109.
14. Liu, H. Q.; Zhao, Y. C.; Cheng, S.; Huang, N.; Leng, Y. X. *J. Appl. Polym. Sci.* **2012**, *124*, 2641.
15. Marziyeh, R. M.; Hajir, Bahrami, S. H.; Arami, M. *J. Appl. Polym. Sci.* **2013**, *129*, 707.
16. Li, Z. H.; Yang, F.; Yang, R. D. *J. Appl. Polym. Sci.* **2015**, *132*, 42663.
17. Hassan, M. M. *RSC Adv.* **2015**, *55*, 35497.
18. Fernández, I. C. S.; Van der Mei, H. C.; Lochhead, M. J.; Grainger, D. W.; Busscher, H. J. *Biomaterials* **2007**, *28*, 4105.
19. Perrin, F. X.; Nguyen, T. D. H.; Nguyen, D. L. *Prog. Org. Coat.* **2015**, *88*, 92.
20. Wang, Q. C.; Uzunoglu, E.; Wu, Y.; Libera, M. *ACS Appl. Mater. Int.* **2012**, *44*, 2498.
21. Ding, X.; Yang, C.; Lim, T. P.; Hsu, L. Y.; Engler, A. C.; Hedrick, J. L.; Yang, Y. Y. *Biomaterials* **2012**, *33*, 6593.
22. Hu, X. F.; Ji, J. *Biomacromolecules* **2011**, *1212*, 4264.
23. Liu, X. S.; Huang, H. Y.; Liu, G. Y.; Zhou, W. B.; Chen, Y. H.; Jin, Q.; Ji, J. *Nanoscale* **2013**, *55*, 3982.
24. Lee, H. J.; Song, J. Y.; Kim, B. S. *J. Chem. Technol. Biotechnol.* **2013**, *88*, 1971.
25. Jiang, S.; Cao, Z. *Adv. Mater.* **2010**, *22*, 920.
26. Li, M.; Neoh, K. G.; Xu, L. Q.; Wang, R.; Kang, E. T.; Lau, T.; Olszyna, D. P.; Chiong, E. *Langmuir* **2012**, *28*, 16408.
27. Razatos, A.; Ong, Y. L.; Boulay, F.; Elbert, D. L.; Hubbell, J. A.; Sharma, M. M.; Georgiou, G. *Langmuir* **2000**, *16*, 9155.
28. Hong, K. H.; Sun, G. *J. Appl. Polym. Sci.* **2010**, *115*, 1138.