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Preparation and Characterization of Chlorinated Cross-Linked Chitosan/Cotton Knit for Biomedical Applications

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Abstract: A new cross-linked chitosan/cotton knit was prepared by the chlorination of chitosan under acidic conditions, and its active chlorine content, mechanical and antimicrobial properties, and rechargeability were characterized. The active chlorine content of the chitosan/cotton knit increased with the concentration of chitosan or sodium hypochlorite used for the treatment and showed a slight decrease upon repeated laundering. The cross-linked chitosan/cotton knit displays lower stress and higher strain than pristine cotton knit. The chlorination of the chitosan/cotton knit resulted in powerful antimicrobial activity against both gram-negative and gram-positive bacteria. For up to 30 days of storage the knit showed excellent rechargeability, taking up chlorine to nearly initial levels. Scanning electron microscope (SEM) observations indicated that chlorination did not cause the damage to the cross-linked chitosan/cotton knit.

Keywords: chitosan, cotton knit, *N*-halamine, active chlorine content, antimicrobial activity.

Introduction

Bacterial contamination of fabric is an important issue in the medical industry, causing infections in patients and employees; cross-infections can occur because of contamination of carpet, beds, accessories, and other items. Hospital gowns and uniforms are an important defense against bacteria, but these items are not foolproof.[1-8]

N-Halamines contain one or more nitrogen-halogen covalent bonds formed by the halogenation of imides, amides, or amines. *N*-Halamines have shown almost instant biocidal activity against a wide range of micro-organisms without causing environmental concerns. It is generally believed that the biocidal action of *N*-halamines is caused by the transfer of positive halogens from *N*-halamines to the appropriate receptors in the cell. This process can effectively destroy or inhibit enzymatic or metabolic cell processes, causing cell death. The active chlorines consumed during halogen transfer can be recharged with chlorine from a subsequent bleach treatment.[9-13]

Liu *et al*. studied the grafting of amide monomers onto cotton cellulose and the subsequent chlorination of the grafted materials. The chlorinated acyclic *N*-halamine cotton cellulose provided rapid inactivation against *Escherichia coli* (a gram-negative bacterium).[14] Ren *et al.* demonstrated that an antimicrobial coating of an *N*-halamine biocidal monomer could be applied to cotton fibers *via* admicellar polymerization. After chlorination with sodium hypochlorite solution, the polymer-coated cotton fabrics effectively inactivated both *Staphylococcus aureus* (a gram-positive bacterium) and *E. coli* after relatively brief contact times.[15] Antimicrobial *N*-halamine-modified chitosan films were investigated by Li *et al*., who found that the chlorinated films showed good efficacy against the two bacterial species (*S. aureus* and *E. coli*) with log reductions of 7.4 and 7.5 within 10 and 5 min of contact time, respectively.[16] The preparation, characterization, and antimicrobial function of *N*-halamine-based chitosan were further examined by Cao *et al*., who transformed the amino groups in chitosan into *N*-halamine structures by chlorine bleach treatment.[17]

In this study, a cross-linked chitosan/cotton knit was prepared by padding amine group-functionalized cross-linked chitosan onto a cotton knit, and subsequently chlorinating the chitosan/cotton knit *via N*-halamine to improve its antimicrobial activity. The chlorinated cross-linked chitosan/cotton knit was characterized with respect to its mechanical strength, surface properties, antimicrobial activity, and rechargeability.

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Experimental

Materials. Chitosan with a deacetylation degree of 90% and a molecular weight of 2,700 g/mol was purchased from Junsei Chemical Co., Ltd. Sodium hypochlorite solution (8%), citric acid, potassium iodide, and sodium hypophosphite were supplied by Aldrich Chemical.

Cross-Linking of Chitosan. Chitosan and epichlorohydrin (in a molar ratio 1:0.5) were added to a 0.067 M NaOH solution and reacted at 40 °C for 4 h. After the reaction, the product was filtered, washed with deionized water several times, and dried at room temperature for 24 h. The cross-linking reaction of chitosan with epichlorohydrin is shown in Scheme I.

Preparation of a Cross-Linked Chitosan/Cotton Knit. Chitosan was dissolved in a 1% acetic acid solution at room temperature for 3 h, and 3% citric acid and sodium hypophosphite were subsequently added to the solution. The cotton knit was soaked in the chitosan solution at room temperature for 1 h. After the padding treatment, the knit was dehydrated up to a weight pick-up of 80%±5% and subsequently subjected to heat treatment at 150 °C for 3 min.

Chlorination of the Cross-Linked Chitosan/Cotton Knit. The pH of a sodium hypochlorite solution was adjusted to 5 by using hydrochloric acid prior to immersing the cross-linked chitosan/cotton knit in the solution at room temperature for 30 min. After chlorination, the knit was rinsed several times with deionized water, and then dried at room temperature for 24 h. The chlorination reaction of cross-linked chitosan is shown in Scheme I.

Measurement of Active Chlorine. The active chlorine content of the chlorinated cross-linked chitosan/cotton knit was measured by iodine titration. About 0.05 g of the cotton knit was dispersed in 40 mL of 1% acetic acid solution, and 1 g of potassium iodide was added. The mixture was stirred vigorously at room temperature for 1 h. The released iodine was titrated with 0.01 mol/L standard sodium thiosulfate solution. The active chlorine content was calculated according to eq. (1):

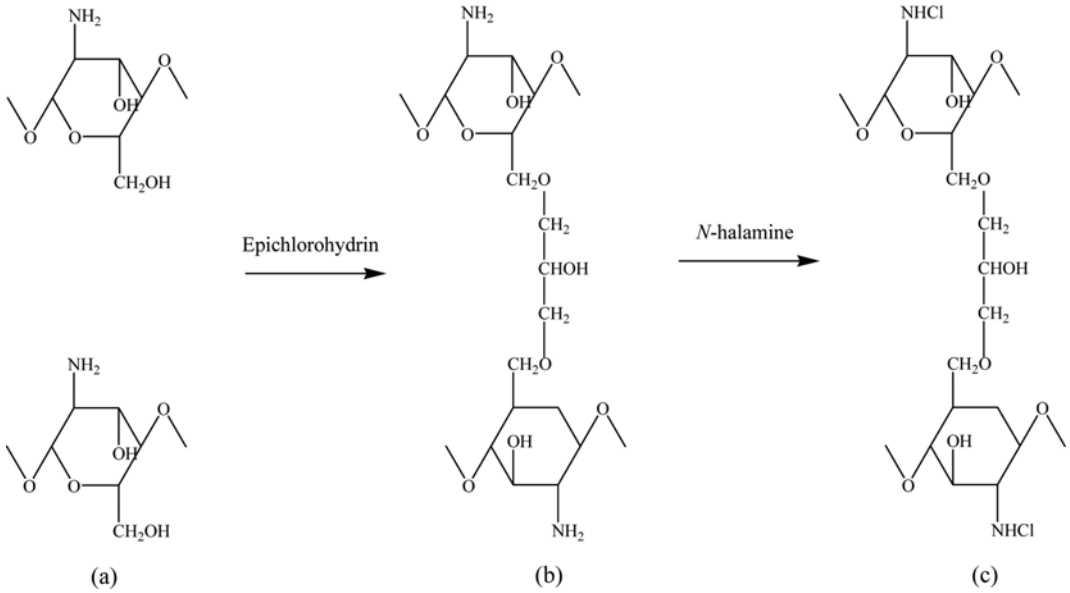
$$Cl = \frac{35.3}{2} \times \frac{(V_{Cl} - V_0) \times 10^{-3} \times 0.01}{W_{Cl}} \times 100 \quad (1)$$

where V_{Cl} and V_0 are the volumes of sodium thiosulfate solution consumed during the titration of chlorinated and nonchlorinated knits, respectively, and W_{Cl} is the weight of the chlorinated cotton knit.

Mechanical Properties. The tensile strength of the cross-linked chitosan/cotton knit, before and after chlorination, was measured using a universal tester (H10KS; Hounsfield Test Equipment, Ltd.) at a cross-head speed of 200 mm/min. All tensile properties were obtained by averaging seven experimental values.

Launderability. A laundering test of the chitosan/cotton knit was performed using a neutral detergent (0.15%) according to the JIS L 0217 method. One cycle of washing included the following steps: the samples were washed for 5 min, dehydrated for 2 min, rinsed for 2 min, dehydrated for 2 min, rinsed for 2 min, dehydrated for 2 min, and finally dried at room temperature for 24 h.

Storage Stability and Rechargeability. The active chlorine content was measured by iodine titration at 25 °C and at a relative humidity of 65% to characterize the storage stability of the chitosan/cotton knit as a function of storage time and recharging. After 30 days, the chitosan/cotton knit was



Scheme I. Cross-linking and chlorination reactions of chitosan with epichlorhydrin and *N*-halamine: (a) chitosan; (b) cross-linked chitosan; (c) chlorinated cross-linked chitosan.

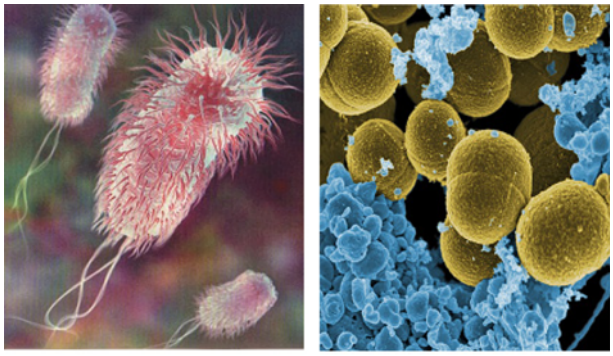


Figure 1. Microphotographs of *E. coli* and *S. aureus*.

successfully rechlorinated under the recharging conditions (0.8% sodium hypochlorite solution, pH 5, and chlorination time of 30 min).

Antimicrobial Properties. The antimicrobial properties of the chitosan/cotton knit against *E. coli* and *S. aureus* were examined according to American Association of Textile Chemists and Colorists (AATCC) test method 100. A liquid cell culture was prepared, transferred into two flasks, and sterilized. The two bacterial species were added, and the solutions were cultivated for 24 h. The cultivated Gram's solution was diluted to 10^5 CFU/mL by using sterilized physiological saline. A solid cell culture was prepared, and Gram's solution was inoculated with the culture. The culture was cultivated at 37 °C for 12 h, and the growth inhibition zones produced by the chlorinated samples were studied. Microphotographs of *E. coli* and *S. aureus* cultures are shown in Figure 1.

Surface Properties. The surface morphology of the chitosan/cotton knit was investigated using a scanning electron microscope (SEM; JEOL JXA840A).

Results and Discussion

Active Chlorine Content. Figure 2 shows the effect of chlorination time on the active chlorine content of the chlorinated cross-linked chitosan/cotton knit at pH 5 and at a sodium hypochlorite concentration of 0.8%. The active chlorine content of the chitosan/cotton knit was lower than 0.25%, but it increased with increasing concentration of the chitosan solution. A slight increase with chlorination time was also evident. Thus, the active chlorine in the cotton knit confers antimicrobial activity against *E. coli* and *S. aureus*.[14,17]

Figure 3 shows the effect of sodium hypochlorite concentration on the active chlorine content of the chlorinated cross-linked chitosan/cotton knit at pH 5 and after a chlorination time of 30 min. The active chlorine content of the chitosan/cotton knit increased with increasing concentration of the sodium hypochlorite solution. Sodium hypochlorite concentration of 0.1% yielded an active chlorine content of

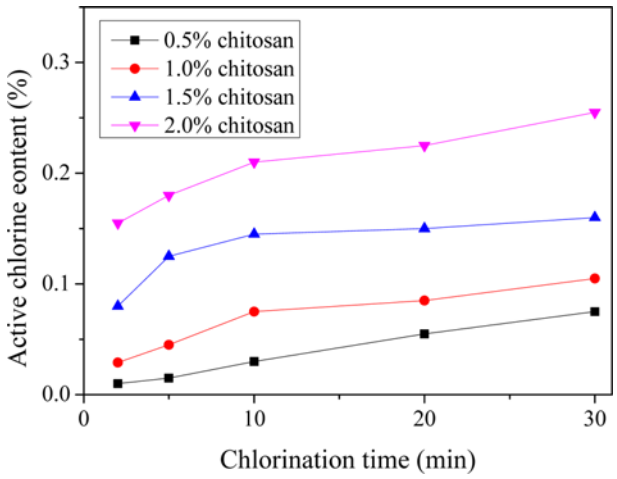


Figure 2. Effect of chlorination time on active chlorine content of chlorinated cross-linked chitosan/cotton knit.

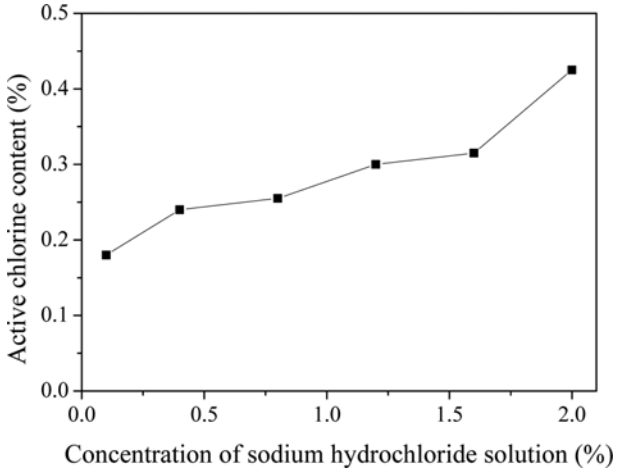


Figure 3. Effect of concentration of sodium hypochlorite solution on active chlorine content of chlorinated cross-linked chitosan/cotton knit.

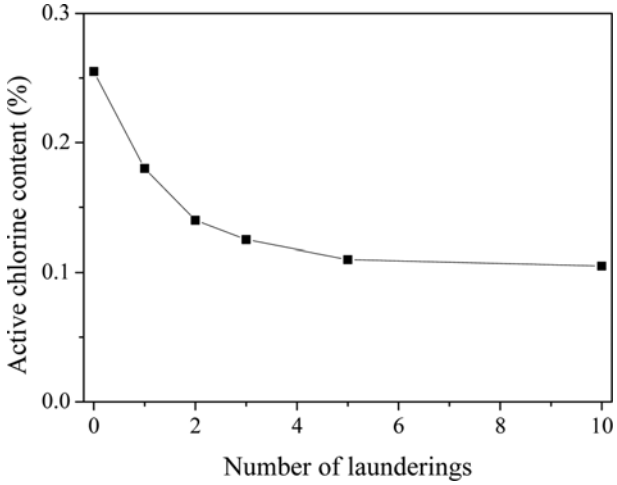
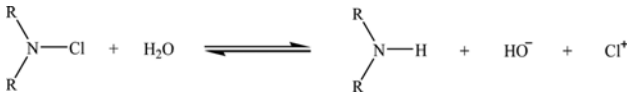


Figure 4. Effect of laundering on active chlorine content of chlorinated cross-linked chitosan/cotton knit.



Scheme II. Dissociation of *N*-halamine to the equilibrium.

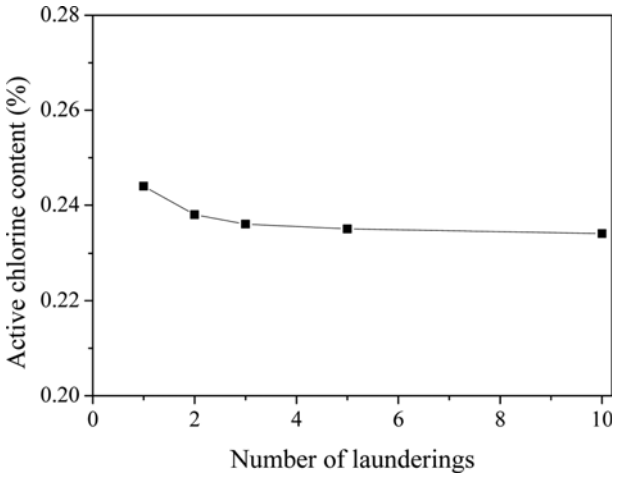


Figure 5. Re-chargeability of active chlorine of chlorinated cross-linked chitosan/cotton knit after laundering.

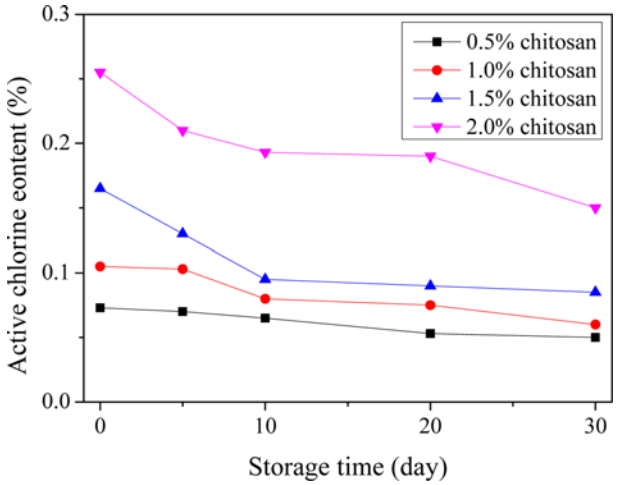


Figure 6. Storage stability of active chlorine for chlorinated cross-linked chitosan/cotton knit at temperature of 25 °C and relative humidity of 65%.

0.18%, while 2% yielded an active chlorine content of 0.42%.

Launderability and Rechargeability. Figure 4 shows the effect of laundering in a neutral detergent on the active chlorine content of the chlorinated cross-linked chitosan/cotton knit. A single cycle of laundering decreased the active chlorine content by 27% to 0.19%. Two cycles of laundering decreased it by 45% to 0.14%. Increasing the number of launderings cycles yielded a further slight decrease in the active chlorine content of the chitosan/cotton knit, likely because of the conversion of the N-Cl covalent bond to HOCl, as shown in Scheme II.[18]

N-Halaminated chitosan fabrics have the outstanding ability to be recharged with active chlorine after it is lost by laundering and antimicrobial action. Figure 5 shows the rechargeability of active chlorine for the chlorinated cross-linked chitosan/cotton knit after laundering. The amount of active chlorine after recharging is similar to the amount after first chlorination, which indicates that the chitosan/cotton knit has excellent rechargeability.

Storage Stability and Rechargeability. Figure 6 shows the storage stability of active chlorine for the chlorinated cross-linked chitosan/cotton knit. Increasing the storage time decreased the active chlorine content of chitosan/cotton knit samples prepared with various concentrations of chitosan. After 30 days, the active chlorine content decreased by about 50%, compared with its initial level. This is because of the dissociation of *N*-halamine by hydrophilic chitosan.

This decrease in active chlorine content over time affects the antimicrobial activity of the chlorinated chitosan/cotton knit. After 20 days, a sample whose initial active chlorine content was 0.5% did not produce a growth inhibition zone for *E. coli* or *S. aureus*, while samples with initial active chlorine contents of 1.5% and 2.0% maintained sufficient active chlorine content to form growth inhibition zones against both bacteria even after 30 days.[15]

The rechargeability of the chlorinated cross-linked chitosan/cotton knit after 30 days of storage was tested under recharging conditions of 0.8% sodium hypochlorite solution, pH 5, and 30 min of chlorination. Upon recharging, the active chlorine content of the rechlorinated cross-linked chitosan/cotton knit was 0.07%, 0.1%, 0.17%, and 0.2% for various concentrations of chitosan. These levels are similar to those measured after initial chlorination, indicating the high rechargeability of the samples after 30 days of storage.

Mechanical Properties. Table I shows the tensile stress and strain of the pristine knit and the cross-linked chitosan/cotton knit. The cross-linked chitosan/cotton knit has lower stress and higher strain compared with the pristine knit, indicating damage due to the high temperature during the heat treatment of the chitosan/cotton knit.[19-21]

The tensile stress and strain of the rechlorinated cross-linked chitosan/cotton knit after ten cycles of laundering are

Table I. Tensile Strain and Stress Values of Pristine Cotton Knit and Chlorinated Cross-Linked Chitosan/Cotton Knit

Sample[a]	Stress (kg/cm)	Strain (%)
a	4.8	305
b	4.0	338
c	3.8	333
d	3.6	328

[a]Note: (a) pristine cotton knit, (b) cross-linked chitosan/cotton knit, (c) chlorinated cross-linked chitosan/cotton knit, (d) rechlorinated cross-linked chitosan/cotton knit after laundering ten cycles.

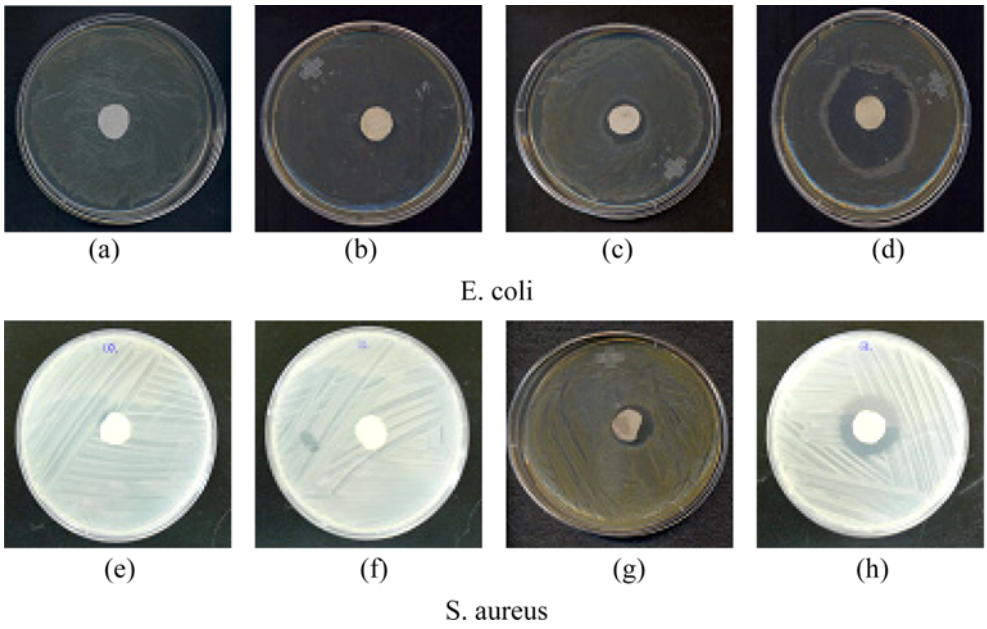


Figure 7. Antimicrobial properties of chlorinated cross-linked chitosan/cotton knit with various chitosan contents: (a) 0.5%, (b) 1.0%, (c) 1.5%, (d) 2.0%, (e) 0.5%, (f) 1.0%, (g) 1.5%, and (h) 2.0% chitosan.

slightly lower than those after first chlorination treatment. This may be attributed to the acidic conditions under which rechlorination was performed. However, the decrease in stress and strain is not significant because the cotton knit was padded with cross-linked chitosan.

Antimicrobial Properties. Figure 7 shows the antimicrobial properties of the knit samples, assessed by measuring the zones of growth inhibition. The pristine cotton knit did not produce a growth inhibition zone in case of *E. coli* or *S. aureus*. The cotton knit padded with cross-linked chitosan and treatment with citric acid instead of chlorine also do not form growth inhibition zones for *E. coli* or *S. aureus*. However, a zone of growth inhibition can be observed for both *E. coli* and *S. aureus* in case of post-chlorination samples of 0.5% and 2% chitosan-padded cotton knits.[22,23]

Table II shows the zones of growth inhibition produced by the chitosan/cotton knit before and after chlorination. As shown in Table II, the 1.5% chitosan-containing cross-linked chitosan/cotton knit forms a zone of growth inhibition measuring 6.5 mm in diameter for *E. coli* and *S. aureus*. The 2% chitosan-containing chitosan/cotton knit produces a zone of growth inhibition measuring 15-21 mm for *E. coli* and 10-13 mm for *S. aureus*. These results also show that chlorinated cross-linking chitosan/cotton knit has higher antimicrobial activity against *E. coli* than against *S. aureus*.

Table II. Growth Inhibition Zone (mm) of Cross-Linked Chitosan/Cotton Knit for *E. coli* and *S. aureus*[a]

E. coli				*S. aureus*			
Un-Chlorination		Chlorination		Un-Chlorination		Chlorination	
a	b	c	d	e	f	g	h
0	0	3-6.5	15-21	0	0	1-5	10-13

[a]Note: (a) 0.5% chitosan, (b) 1.0% chitosan, (c) 1.5% chitosan, (d) 2.0% chitosan, (e) 0.5% chitosan, (f) 1.0% chitosan, (g) 1.5% chitosan, (h) 2.0% chitosan.

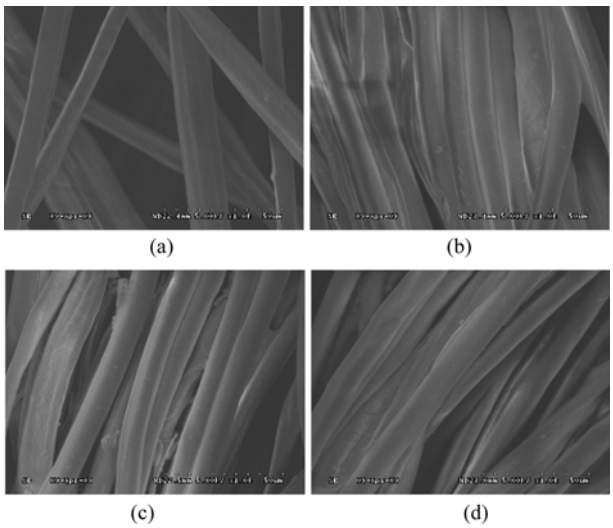


Figure 8. Scanning electron microscope photographs of pristine cotton knit and chlorinated cross-linked chitosan/cotton knit: (a) pristine cotton knit, (b) cross-linked chitosan/cotton knit, (c) chlorinated cross-linked chitosan/cotton knit, and (d) rechlorinated cross-linked chitosan/cotton knit after laundering ten cycles.

Surface Properties. Figure 8 shows SEM images of the pristine knit (Figure 8(a)) and the cross-linked chitosan/cotton knit before chlorination (Figure 8(b)), after chlorination

(Figure 8(c)), and after laundering and rechlorination (Figure 8(d)). Figure 8(b) shows that the cross-linked chitosan is uniformly padded on the cotton knit. Figure 8(c) shows that the chitosan is not damaged by sodium hypochlorite during chlorination treatment. Figure 8(d) is an image of the rechlorinated cross-linked chitosan/cotton knit after ten cycles of laundering, which shows that the cross-linked chitosan does not detach from the cotton knit and that it is not damaged by sodium hypochlorite during chlorination.[24]

Conclusions

We report a simple and practical approach by which active chlorine was introduced into chitosan derivatives, which formed a padding layer around cotton knit fibers, to dramatically reinforce the antimicrobial activity of the original cotton knit. Chlorinating the cross-linked chitosan/cotton knit under acidic conditions with diluted sodium hypochlorite solution produced the highest. The chlorinated chitosan/cotton knit exhibited powerful antimicrobial activity against both gram-negative and gram-positive bacteria. After the active chlorine was consumed, the chitosan/cotton knit exhibited excellent rechargeability; it was able to take up chlorine to nearly initial levels. SEM images of the surface structure showed that the chitosan/cotton knit was not damaged under the acidic conditions of treatment. These encouraging results indicate that *N*-halamine-based chitosan derivatives may have a wide range of applications in biomedicine and health care, including wound dressings, coatings for medical devices, and hospital linens.

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