The Preparation and Characterization of Chitosan Wound Dressings with Different Degrees of Acetylation

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ABSTRACT: Chitosan fibers have in recent years found applications in various fields such as antimicrobial textiles and wound dressings. In this study, chitosan fibers with different degrees of acetylation were prepared by controlling the ratio between the amount of acetic anhydride and the weight of the fibers during the acetylation process. The absorption and antimicrobial properties, as well as the dry and wet strength of nonwoven chitosan wound dressings with different degrees of acetylation were studied. Results showed that the partially acetylated chitosan wound dressing had a much higher absorption capacity than the original untreated chitosan samples, while there was a reduction in the wet strength and antimicrobial property for the partially acetylated chitosan nonwoven dressing. © 2007 Wiley Periodicals, Inc. J Appl Polym Sci 107: 993–999, 2008

Key words: biopolymer; chitosan fiber; wound dressing; antimicrobial property

INTRODUCTION

Chitin, poly-(1 → 4)-2-acetamido-2-deoxy-β-D-glucose, is the second most abundant natural polymer. Chitosan is the deacetylated product of chitin, i.e., poly-(1→4)-2-amino-2-deoxy-β-D-glucose.[1] While chitin is difficult to dissolve in most common solvents, chitosan is easily soluble in aqueous acidic solutions and chitosan fibers can be readily made in a wet spinning process.[2,3] As a novel textile fiber, chitosan fibers are now widely used in the production of underwear clothing, where its high moisture absorption and broad spectrum antimicrobial properties are particularly useful. In the healthcare and medical industry, chitosan fibers have been made into nonwoven wound dressings and 3D structures used in the manufacture of artificial scaffold, utilizing their biocompatibility, biodegradability, nontoxicity and the ability to promote wound healing.[4]

With chitosan fibers, the degree of acetylation (DA) on the C2 amine groups in the glucosamine residues is one of the most important factors affecting its physical properties. In recent years, much work has been carried out to prepare chitosan with different DA to assess the effect of DA on the biomedical properties.[5–8] Chen and Hua[9] studied the effect of N-acetylation on the acidic solution stability, thermal, and mechanical properties of membranes prepared from chitosan. After assessing a series of chitosan membranes with different degrees of N-acetylation, they found that the differences in acidic solution stability and thermal and mechanical properties were attributed to differences in crystallinity of the membrane as the result of preparation conditions and N-acetylation. Results showed that after N-acetylation, the acidic solution stability of N-acetylated membranes increased and the tensile strengths became higher, while the swelling index also increased.

Ren et al.[10] prepared chitosan matrices by compression molding and investigated the degradation of chitosan with different degrees of N-acetylation. Results showed that the initial degradation rate, equilibrium water absorption, and the degree of swelling increased with increasing DA. Wenling et al.[11] found that the swelling index of chitosan films decreased and their elastic modulus and tensile strength increased with the decrease in DA. Je et al.[12] found that cytotoxic activities of the chitosan derivatives were dependent on their DA.

Cho et al.[13] prepared partially acetylated chitosans with different degrees of acetylation by alkaline treatment of chitin under homogeneous conditions. The chitosan sample became soluble in dilute acetic acid at a DA of about 72% or over and soluble in water at a DA of about 51%. The solubility of the partially acetylated chitosans had a close relationship with their crystal structure, crystallinity, and crystal imperfection as well as the glucosamine content. Wide-angle X-ray diffractometry studies revealed

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TABLE I
Treatment Conditions for Chitosan Wound Dressings with Different Degrees of Acetylation

Sample	Weight of chitosan nonwoven felt (g)	Amount of methanol (mL)	Amount of water (mL)	Amount of acetic anhydride (mL)
1	3.126	100	1	0
2	2.987	100	1	1.8
3	3.232	100	1	4.0
4	3.107	100	1	15.0

that the sample with 72% DA retained the crystal structure of α-chitin with significantly reduced crystallinity and perfection of the crystallites. The water-soluble sample of 51% DA had a new crystal structure similar to that of β-chitin rather than either α-chitin or chitosan, suggesting that the homogeneous deacetylation transformed the crystal structure of chitin from α to β form. Some hydrogen bonds existing in raw α-chitin were found to be missing at a DA of 51%. Other studies also showed that when chitosan was half acetylated with acetic anhydride, it became water soluble.[14,15]

The present work studied the effect of acetylation on the properties of chitosan wound dressings. Nonwoven chitosan fabrics were treated with different amount of acetic anhydride to prepare chitosan wound dressings with different degrees of acetylation. The absorption and antimicrobial properties of the resultant samples were analyzed.

EXPERIMENTAL

The chitosan nonwoven samples were purchased from Haixiao Ltd (Qingdao, China). It is a needle punched fabric with a weight per unit area of about 100 g/m^2. This sample was washed with deionized water and acetone before it was used in the present study.

Acetylation of the chitosan nonwoven samples was carried out with acetic anhydride in methanol. Four lots of chitosan nonwoven samples, each weighing about 3 g, were first cut to 5 cm × 5 cm sizes and placed in a 250-mL conical flasks. Methanol (100 mL) and 1 mL water were then added into the flasks before different amounts of acetic anhydride were added into the flasks. The samples were placed under room temperature for 24 h before being washed with 4% aqueous NaOH solution for 24 h at room temperature. The nonwoven samples were then washed with deionized water three times before being washed with acetone and dried at 60°C. Table I shows the detailed treatment conditions.

The DA was analyzed by using elemental analysis. Since the chemical formula for chitosan is $C_6NO_4H_{11}$ and that of chitin with 100% acetylation is $C_8NO_6H_{13}$, the ratio between N/C contents can be used to calculate the DA. Assuming X% of the amine groups are acetylated, then:

$$r = \mathrm{N}/[6\mathrm{C}(1-X\%) + 8\mathrm{C}\ X\%] = \mathrm{N}/[6\mathrm{C} + 2\mathrm{C}\ X\%]$$

$$X = (\mathrm{N}/r - 6\mathrm{C})/2\mathrm{C} = (14.007/r - 72.066)/24.022 \times 100$$

When testing the absorption properties of the chitosan samples, a piece of 5 cm × 5 cm dressing was weighed first (W) before being placed in a petri dish 90 mm in diameter, and wetted with 40 times its own weight of solution A (an aqueous solution containing 142 mmol of sodium chloride and 2.5 mmol of calcium chloride). The dish was then placed in a 37°C oven for 30 min. After that, the dressing was lifted out of the solution by holding it with a forcep at one corner. The solution was left to drip for 30 s and the wet dressing was weighed (W_1). The sample was then wrapped in a piece of polyester cloth and centrifuged for 5 min before being weighed again (W_2). Finally, the dressing was dried to constant weight at 105°C for 4 h and the dry weight was recorded (W_3).

In the above test, $(W_1 - W)/W$ represents the overall absorption capacity of the chitosan wound dressing. The fluid absorbed by the dressing can be further divided into two parts, i.e., those held in the textile structure between the fibers and those held inside the individual fibers. In the aforementioned experiment, $W_1 - W_2$ is the weight of fluid held between the fibers whilst $W_2 - W_3$ is the weight of fluid held within the fibers. The ratio of $(W_1 - W_2)/W_3$ and $(W_2 - W_3)/W_3$ are calculated to convert the fluid absorption into gram fluid absorbed per gram of dry fiber, which can be used to compare different types of dressings.

When testing the antimicrobial effect of the chitosan samples, a strip of agar was removed from a standard agar plate, leaving two areas of agar separated by a narrow channel. During the test, the agar gel on one side of the plate was inoculated with a bacterial suspension. After a short drying period, a strip of the dressing under test, ∼ 10 mm wide and 50 mm long, was placed across the two agar areas forming a bridge. The fluid reservoir between the two sides of the agar gel was then filled with sterile water, and the plate incubated in the upright position for 24 h.

TABLE II
Nitrogen and Carbon Contents and Degree of Acetylation of the Four Nonwoven Chitosan Samples

Sample	Nitrogen content (%)	Carbon content (%)	N/C ratio	Degree of acetylation (%)
1	6.962	38.72	0.1798	24.54
2	6.814	38.99	0.1748	33.90
3	6.800	40.06	0.1697	43.60
4	5.949	40.83	0.1457	100.44

During this period, the agar absorbed some of the added water. This was taken up by the dressing strip and distributed throughout its length, carrying with it bacteria drawn up from the surface of the agar island. In the absence of significant antimicrobial activity within the dressing, these microorganisms would eventually reach the second area of agar, where they would grow to form colonies around the margin of the test sample.

Tensile strength of the chitosan nonwoven dressings were measured using a gauge length of 20 mm and a test speed of 20 mm/min. The chitosan samples were cut to strips with a width of 10 mm and a length of 50 mm. During the test for wet strength, the sample was first wet in solution A for 1 min before the test was carried out.

RESULTS AND DISCUSSION

In a previous study, it has been shown that chitosan fibers can be converted into chitin fibers by treating the fibers with acetic anhydride in methanol. Fibers with different degrees of acetylation can be prepared by controlling the treatment time, temperature, and the ratio between the amount of anhydride and the weight of the fiber.[2] In the present study, four samples of chitosan nonwoven felt were treated with different amounts of acetic anhydride. As can be seen in Table II, chitosan wound dressings with degrees of acetylation of 24.54%, 33.90%, 43.60%, and 100.44% were prepared in this method, with sample 3 approaching the theoretical half chitosan and half chitin. Figure 1 shows the infrared spectroscopy of untreated chitosan and the fully acetylated sample. It is clear that after the acetylation treatment, the amine groups in the chitosan fibers are converted into acetamide groups, resulting in regenerated chitin fibers.

Table III summarizes the various weights recorded for the four samples in the absorption tests. While the original chitosan felt had an absorption capacity of 5.61 g g^{-1}, sample 3, with a DA of 43.60%, had an

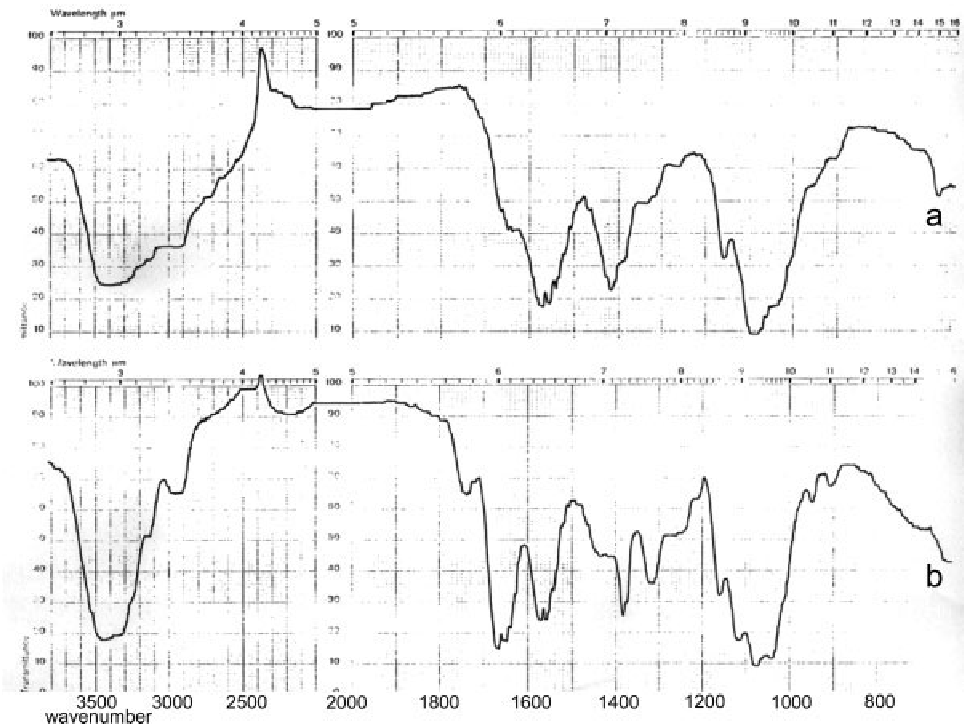

Figure 1 Infrared spectroscopy of chitosan and acetylated chitosan samples, (a) chitosan; (b) fully acetylated chitosan.

TABLE III
Absorption Capacity for Four Chitosan Nonwoven Felt Samples with Different Degrees of Acetylation

Sample	W (g)	W_1 (g)	W_2 (g)	W_3 (g)	$(W_1 - W)/W$ (g g^{-1})	$(W_2 - W_3)/W_3$ (g g^{-1})
1	0.273	1.806	0.407	0.256	5.61	0.59
2	0.236	3.104	0.914	0.209	12.15	3.37
3	0.242	4.069	1.113	0.215	15.81	4.18
4	0.172	2.642	0.345	0.147	14.36	1.35

absorption capacity of 15.81 g g^{-1}, an increase of 181.8%. This increase is significant as it implies that when applied to exuding wounds, the partially acetylated chitosan nonwoven dressing can absorb almost three times more wound exudates as compared with the original chitosan nonwoven sample. These results correspond well to the absorption results reported previously for chitosan films and membrane reported by other researchers. It is an indication that the regular crystalline structure of the chitosan fibers was disrupted by the insertion of acetyl groups, making it easy for water to penetrate into the fiber structure.

Figures 2 and 3 show the effect of the DA on the absorption capacity and gel swelling ratio of chitosan wound dressings respectively. While the absorption capacity peaked at DA close to 50/50, similar results were noted for the gel swelling ratio, which measure ratio between the weight of the swollen chitosan fibers and that of the dry fibers. The original chitosan fibers had a gel swelling ratio of 1.59, while the fiber with a DA of 43.60% had a gel swelling ratio of 5.18, a clear indication that the partially acetylated chitosan fibers can absorb more water into the fiber structure, making nonwoven dressing more absorbent as a result.

Figure 4 shows the photomicrographs of the dry and wet structures of the orginal and partially acetylated chitosan nonwoven dressings. While the original chitosan nonwoven showed a slight degree of wetting when placed in contact with water, the fibers in the partially acetylated chitosan samples swell on contact with water, and as the fibers expanded, the capillary spaces within the nonwoven structure were closed (Fig. 5). This makes it difficult for liquid to migrate along the nonwoven structure, thus blocking the spread of wound exudate to move laterally from the wound surface to the healthy skin surrounding the wound edge, thus helping reduce wound maceration.

Figure 6 shows the load-extension graphs of chitosan nonwoven dressings with different degrees of acetylation. It is clear that while the dry strength were similar for the three samples, the chitosan nonwoven dressing with a DA of 43.60% had a much lower wet strength than the other two samples. As summarized in Table IV, the ratio between the wet and dry strength of the three chitosan nonwoven dressings showed that the partially acetylated chitosan nonwoven dressing had a poor wet strength, indicating the effect of the disruption of the crystalline structure on the physical properties of the non-

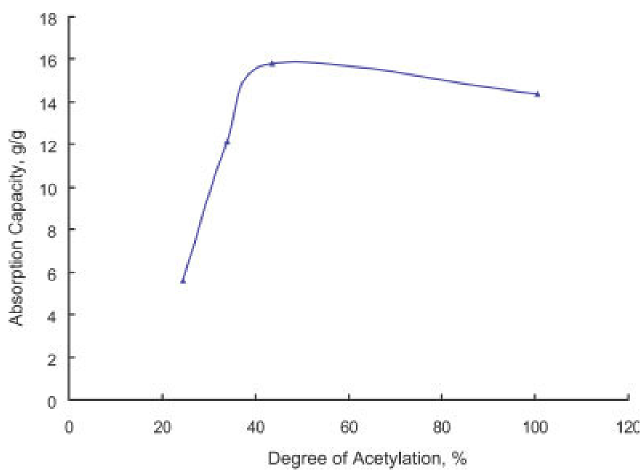

Figure 2 Effect of the degree of acetylation on the absorption capacity of chitosan wound dressings. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

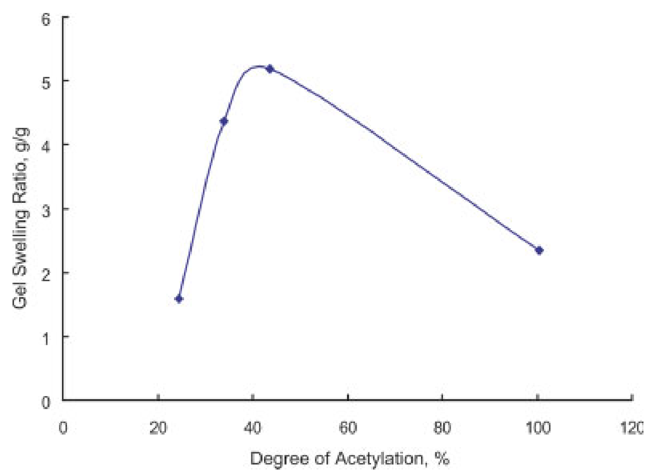

Figure 3 Effect of the degree of acetylation on the gel swelling ratio of chitosan wound dressings. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

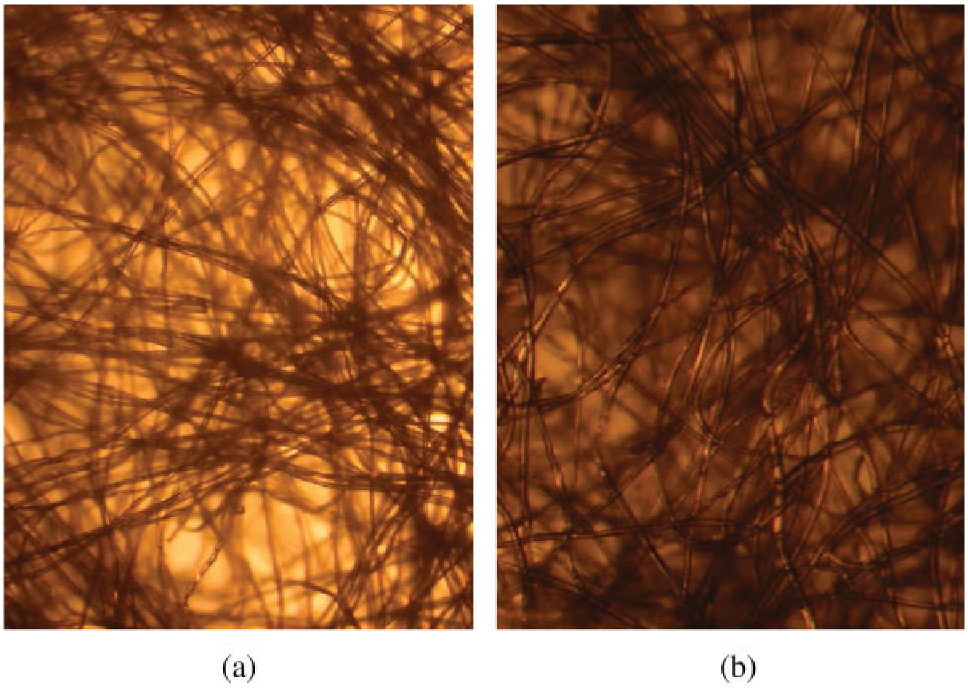

Figure 4 Photomicrographs of the dry and wet structures of the orginal chitosan nonwoven dressings, ×200, (a) dry; (b) wet. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

woven fabric. Clinically, this reduction in felt strength could be beneficial, as it makes it easy to remove the dressing from the wound surface without damaging the underlying tissue.

Figure 7 shows the antimicrobial test results of the chitosan nonwoven dressings with different degrees of acetylation. For the original chitosan sample and the fully acetylated sample, there was no sign of the presence of bacteria on the agar gel across the nonwoven strips, indicating the fact that no bacteria could migrate along the fabric. On the other hand, for the sample with a DA of 43.60%, a cluster of bac-

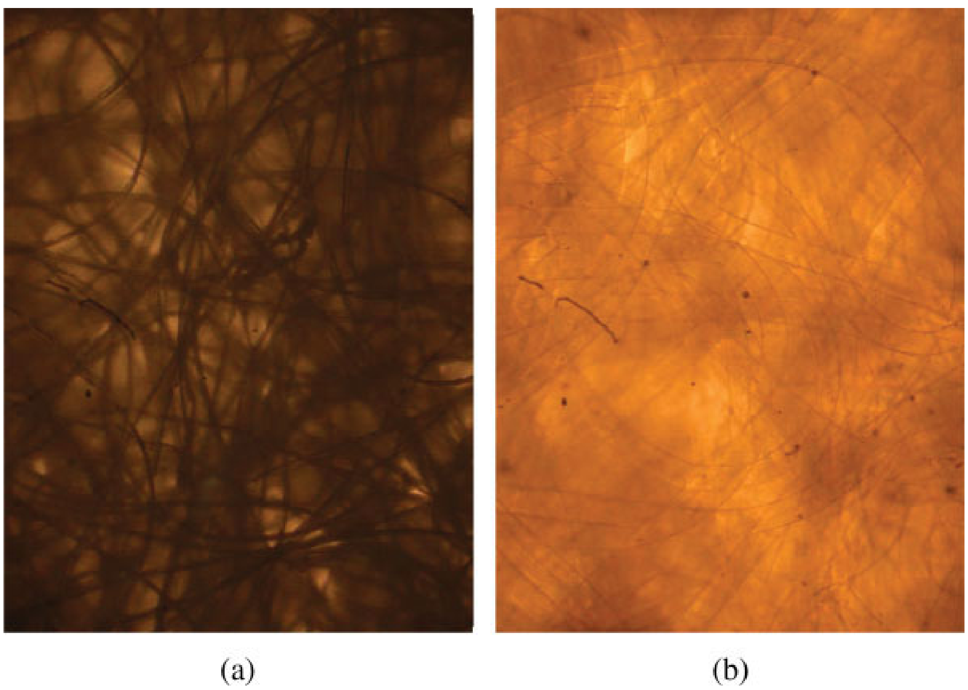

Figure 5 Photomicrographs of the dry and wet structures of the partially acetylated chitosan nonwoven dressings, ×200, (a) dry; (b) wet. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

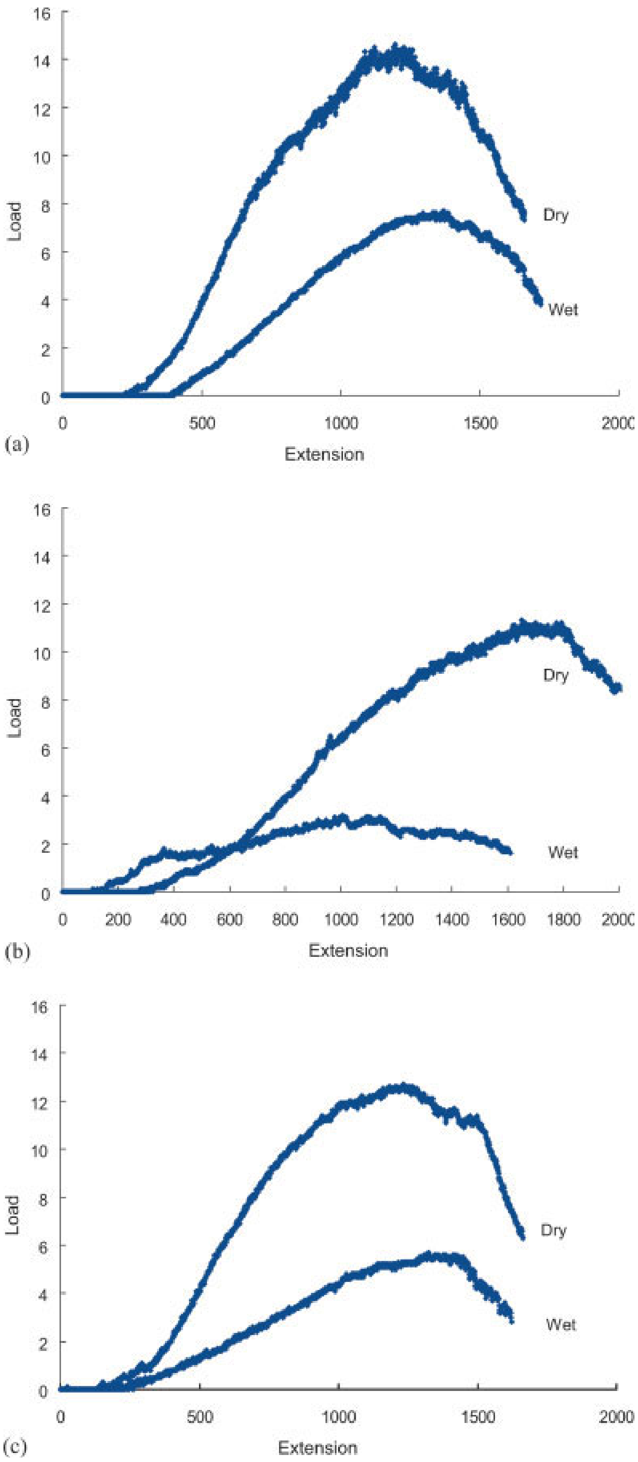

Figure 6 Load-extension graphs of chitosan nonwoven dressings with different degree of acetylation, (a) DA: 24.54%; (b) DA: 43.60%; (c) DA: 100.44%. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

teria can be seen around the end of the nonwoven strip, a clear sign that bacteria had migrated along the fabric during the test. This can be attributed to two possible factors, i.e., the high absorption capacity of the partially acetylated sample, which makes it

TABLE IV
The Dry and Wet Strength of Chitosan Nonwoven Dressings with Different Degree of Acetylation

Degree of acetylation	Dry strength (N)	Wet strength (N)	Ratio between wet and dry strength
24.54%	14.5	7.7	0.53
43.60%	11.3	3.2	0.28
100.44%	12.6	5.7	0.45

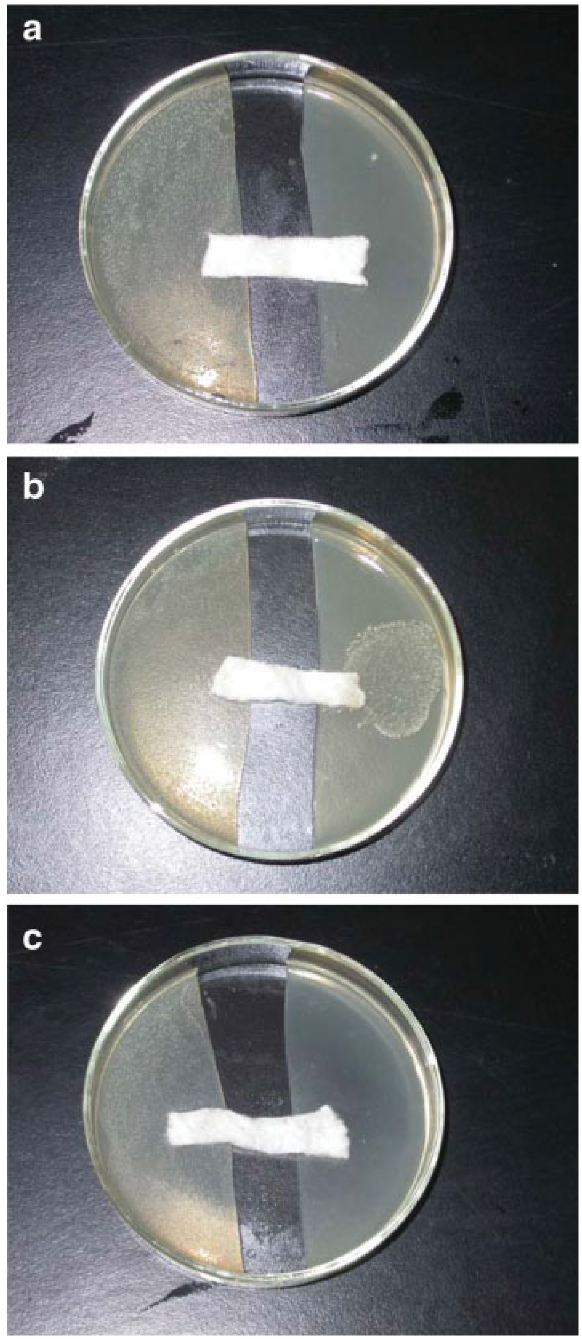

Figure 7 Antimicrobial test result of the chitosan nonwoven dressings with different degrees of acetylation, (a) DA: 24.54%; (b) DA: 43.60%; (c) DA: 100.44%. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

easy for bacteria to move along a wet structure, and the reduced antimicrobial properties because of the disruption of the regular fiber structure.

CONCLUSIONS

This study showed that chitosan wound dressings with different degrees of acetylation can be prepared by treating chitosan nonwoven fabric with acetic anhydride. There were clear differences in the absorption and antimicrobial properties between the samples with different degrees of acetylation. Partially acetylated chitosan nonwoven dressings had a higher absorption capacity, a lower wet strength and a reduced antimicrobial ability than the original and the fully acetylated samples. Test results showed that the partially acetylated chitosan wound dressings can absorb a large amount of water into the fiber structure, hence reducing the capillary space of the nonwoven dressing and making it difficult for liquid to migrate laterally along the fabric structure.

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