The Absorption and Release of Silver and Zinc Ions by Chitosan Fibers

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ABSTRACT: When chitosan fibers were treated with $AgNO_3$ and $ZnCl_2$ solutions, the silver and zinc ions were chelated by chitosan through the amine groups in the fibers. These novel metal ions can be released into the solution when the silver- and zinc-containing fibers are placed in contact with normal saline. Results showed that the silver-containing chitosan fibers have good antimicrobial properties, while the zinc-containing fibers can be used to deliver zinc ions in wound care applications. © 2006 Wiley Periodicals, Inc. J Appl Polym Sci 101: 766–771, 2006

Key words: chitosan fiber; silver ions; zinc ions; antimicrobial

INTRODUCTION

In recent years, chitosan has found widespread uses as a novel biomaterial.[1–5] As a natural polymer, chitosan is biocompatible, biodegradable, and nontoxic. As a renewable resource, it has abundant supplies in nature. Fibers made from chitosan possess both the novel bioactivities of chitosan and the good processibilities of a fibrous material.[6–8] In recent years, woven, nonwoven, and knitted structures of chitosan fibers have been made and used in wound care and other biomedical fields.

Because of the primary amine groups in the structure, chitosan is a natural chelating polymer with excellent absorption capacities for copper, zinc, silver, and many other heavy metal ions.[9–11] This absorption of metal ions can occur in solid state, especially with chitosan fiber, as it has a large specific surface area. A previous study has shown that chitosan fibers can chelate up to 9.0% and 6.2% of their own weight of copper and zinc ions, respectively.[11]

Many heavy metal ions are useful in biomedical applications. For example, silver ions, while possessing minimal toxicity to human body, have excellent antimicrobial properties.[12–20] Zinc ions are required in the proper functioning of many human enzymes, hence, topical or systemic delivery of zinc ions is necessary for zinc deficient patients.[21] For burn wounds, where the loss of zinc ions is common, delivery of zinc through the use of appropriate zinc-containing dressings is often used.

This study evaluated the possibility of using chitosan fibers as carriers of silver and zinc ions. The two metal ions were first chelated to the fibers by treating the chitosan fibers with aqueous solutions of $AgNO_3$ and $ZnCl_2$. The release of silver and zinc ions into normal saline was also studied.

EXPERIMENTAL

The chitosan fibers were made by extruding a 5% wt/wt chitosan solution dissolved in 2% aqueous acetic acid into a 5% aqueous NaOH solution. The fibers were stretched in a hot water bath before they were washed and dried. The spinneret used had a diameter of 80 μm, and the fiber diameter was about 25 μm. The degree of deacetylation for the chitosan is about 85%.

Chitosan fibers with different silver contents were made by treating the fibers with aqueous solutions containing different amounts of $AgNO_3$. Five samples each weighing 1 g were placed in contact with 50 mL aqueous solutions containing 0.01, 0.02, 0.03, 0.05, and 0.5 g/L $AgNO_3$. The mixtures were left standing for 24 h at room temperature before the fibers were separated from the solutions, washed with distilled water, and dried.

When evaluating the effect of treatment time, four samples each weighing 1 g were placed in contact with 50 mL aqueous solutions containing 0.5 g/L $AgNO_3$. The mixtures were left standing for 15 min, 30 min, 5 h, and 24 h respectively, at room temperature before the fibers were separated from the solutions, washed with distilled water and dried.

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TABLE I
Effect of $AgNO_3$ Concentration on the Silver Content in Chitosan Fibers

	$AgNO_3$ concentration (g/L)				
	0.01	0.02	0.03	0.05	0.5
Fiber silver content (ppm)	5.0	20.6	47.2	69.0	1496.0
Total amount of silver in 50 mL solution (mg)	0.32	0.64	0.95	1.59	15.90
Total amount of silver in 1 g fiber (mg)	0.005	0.0206	0.0472	0.069	1.496
Percentage of silver absorbed by the fiber	1.56	3.22	4.97	4.34	9.4

When analyzing the silver ion contents in the treated fibers, 0.5 g fibers were treated with 7 mL concentrated sulfuric acid until the fibers were fully digested. The mixture was then diluted to 100 mL with distilled water and filtered. The silver ion concentration was determined by using atomic absorption spectrometer.

When testing the release of silver ions from the fibers, 10 g chitosan fibers were first treated with 100 mL aqueous solution containing 0.5 g/L $AgNO_3$ at room temperature for 24 h. The fibers were washed and dried before treated fibers (5.5 g) were placed in contact with 220 mL normal saline (0.9% aqueous NaCl solution). The mixture was conditioned at 37°C and after 0.5, 1, 5, 8, 24, and 48 h respectively, 5 mL solution was taken and tested for silver ion concentration by using atomic absorption spectrometer.

When applying zinc ions onto the chitosan fibers, five samples each weighing 1 g were placed in contact with 100 mL aqueous solutions containing 0, 6, 7, 8, and 8.5 g/L $ZnCl_2$. The mixtures were left standing for 24 h at room temperature before the fibers were separated from the solution. After washing and drying, 0.5 g of the treated samples were digested with 7 mL concentrated sulfuric acid before analyzing the zinc contents by titration with EDTA solutions.

When assessing the effect of time on zinc uptake, five samples each weighing 1 g were placed in contact with 80 mL aqueous solutions containing 10 g/L $ZnCl_2$. The mixtures were left standing for 0.25, 1, 3, 5, and 24 h at room temperature before the fibers were separated from the solution. After washing and drying, 0.5 g of the treated samples were digested with 7 mL concentrated sulfuric acid before analyzing the zinc contents by titration with EDTA solutions.

When testing the release of zinc ions from the chitosan fibers, 3.5 g treated chitosan fibers (with 22.75 mg zinc per gram of fiber) were placed in contact with 140 g 0.9% normal saline solution. After 1, 3, 5, 8, and 24 h, 10 mL solution was taken out and the zinc contents were analyzed by titration with EDTA.

To assess the effect of silver on the antimicrobial properties of the chitosan fibers, to each of four test tubes were added 10 mL 0.5% peptone water and 0.1 mL bacteria suspension with a concentration of 1×10^8 cfu/mL *Escherichia coli* (*E. coli*). Then to three of these tubes were added 0.1 g sterilized viscose rayon fiber, chitosan fiber, and the silver-containing chitosan fiber, respectively. The test tubes were then placed in a 36°C water bath and shaken at a speed of 120 r/min for 12 to 15 h. The antimicrobial efficacy can be judged by the clarity of the solution around the fibers.

The zone of inhibition test was also used to assess the antimicrobial properties of the chitosan fibers qualitatively. Petri dishes containing a 5 mm layer of tryptone soya agar were inoculated with 0.2 mL of a log phase broth culture of *E. coli.* This suspension was distributed uniformly over the surface of the plate and allowed to dry for 15 min. The original chitosan fiber (0.1 g) and silver-containing chitosan fiber were then placed on the agar. The plates containing the bacteria were incubated for 24 h at 35°C. At the end of the incubation period, the plates were examined for microbial growth and the presence of a zone of inhibition.

Quantitatively, the antimicrobial activity of the fibers were tested against three common strains of bacteria, i.e., *E. coli.*, *Staphylococcus aureus* (*S. aureus*) and *Bacillus sabtilis* (*B. sabtilis*). The bacteria were suspended in 0.5% peptone water with the bacteria concentration at about 1.5×10^4 to 1.5×10^5 cfu/mL. Peptone water (35 mL 0.5%) was measured into 100 mL conical flasks and to each of them were added 2.5 mL of the bacteria suspension, with the bacteria concentration in the conical flask controlled at between 1×10^3 and 1×10^4 cfu/mL. After that, 0.375 ± 0.002 g of sterilized viscose rayon fiber, chitosan fiber, and silver-containing chitosan fiber were added into the conical flasks respectively. The test on each type of the fiber and the control were repeated three times. After the fibers were placed in contact with the bacteria suspension, the conical flasks were placed in a 36°C water bath and shaken at a speed of 180 r/min for 8 h. For the control sample and viscose rayon, a sample

TABLE II
Effect of Treatment Time on Fiber Silver Content

	Treatment time			
	15 min	30 min	5 h	24 h
Fiber silver content (ppm)	402.0	502.1	968.2	1496.0

TABLE III
Release of Silver from Silver-Containing Chitosan Fiber

	Contact time (h)					
	0.5	1	5	8	24	48
Silver concentration in contacting solution (ppm)	0.36	0.46	0.61	0.70	1.05	2.81
Milligrams of silver released per gram fiber	0.0145	0.0186	0.0245	0.0279	0.0420	0.112

solution was taken from each flask and diluted with 0.5% peptone water to 10^{-3}, 10^{-4}, and 10^{-5} of the orginal concentration. After that, 0.1 mL solution was taken and placed on tryptone soya agar and incubated for 40–48 h to measure the colony forming units. For the chitosan sample, the solution was diluted with 0.5% peptone water to 10^{-2}, 10^{-3}, and 10^{-4} before 0.1 mL solution was taken and placed on tryptone soya agar and incubated for 40–48 h to measure the colony forming units. For the silver-containing chitosan fiber, 0.1 mL of the bacteria-containing solution was directly used to measure the colony forming units. The reduction in the number of bacteria is calculated in the following equation.

$$Reduction\ in\ bacteria\ =\ [A-B]/A\ \times\ 100\%$$

where A is the average bacteria concentration in the control sample after shaking (cfu/mL); B is the average bacteria concentration in the test sample after shaking (cfu/mL).

RESULTS AND DISCUSSION

Table I shows the effect of $AgNO_3$ concentration on the silver contents of the chitosan fibers. It can be seen that the fiber silver contents increase as the $AgNO_3$ concentration increases. When the $AgNO_3$ concentration was 0.5 g/L, the silver concentration in the fiber reaches 1496.0 ppm, which is equivalent to the silver concentration of Johnson and Johnson's silver-containing Actisorb antimicrobial wound dressings. In a similar work, it was shown that at a silver concentration of about 170 ppm, alginate fibers became highly antimicrobial against a wide range of bacteria.[22] It is, therefore, feasible to incorporate a clinically significant amount of silver ions into the chitosan fibers by a simple treatment of the fibers with an aqueous solution of $AgNO_3$.

The results in Table I showed that during the treatment, only a small amount of the $AgNO_3$ in the solution was absorbed by the fiber. This is understandable as during the treatment, the chitosan fiber was in a solid state. It is important to maintain a relatively high concentration of $AgNO_3$ in the contacting solution in order for the silver ions to diffuse into the chitosan fibers.

(a)

(b)

Figure 1 Zone of inhibition, (a) original chitosan fiber; (b) silver-containing chitosan fiber. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

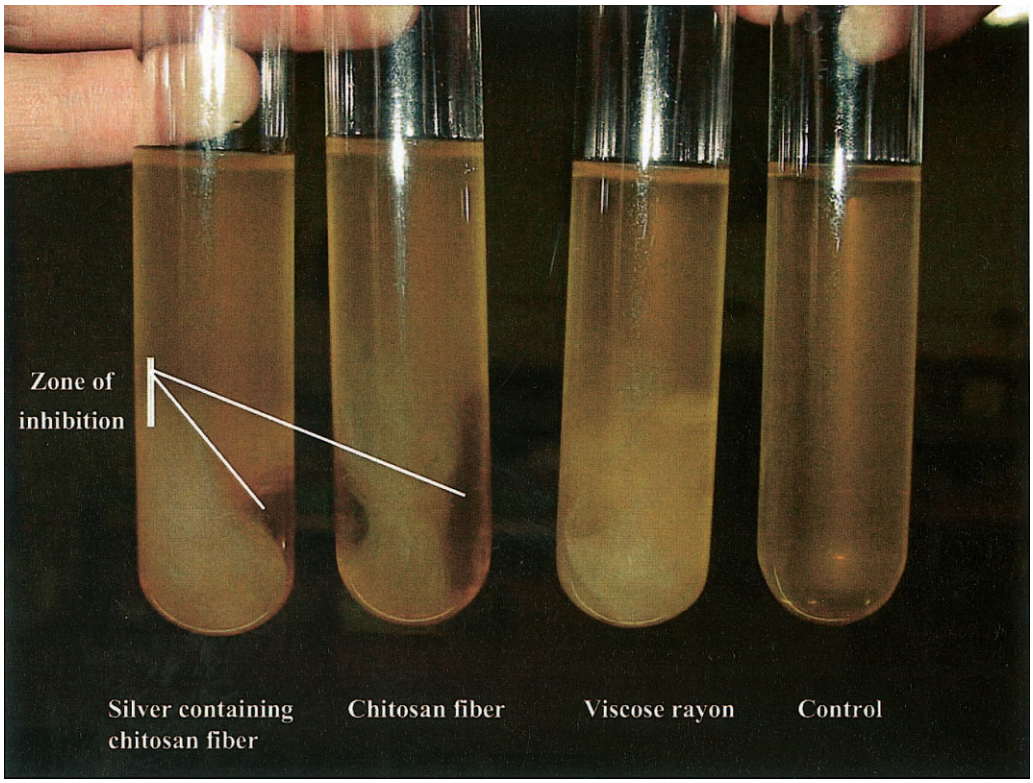

Figure 2 Clarity of solutions in the control sample and in suspension of *Escherichia coli* with silver-containing chitosan fiber, chitosan fiber, and viscose rayon. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Table II shows the effect of time on the absorption of silver ions by chitosan fibers. It can be seen that the absorption of silver ions by chitosan fibers is relatively a slow process. The silver content in the fiber was 402.0 ppm after 15 min of treatment, and it slowly rose to 1496.0 ppm after 24 h. These results showed that in order for the chitosan fiber to contain a sufficient amount of silver ions, the treatment should be carried out over an extended period of time. On the other hand, by controlling the treatment time, it is possible to obtain fibers with different concentrations of silver ions.

As a novel biomaterial, chitosan fibers are particularly suited for the manufacture of wound dressings, where their biocompatible, biodegradable, nontoxic, and highly absorbing properties are particularly useful for wounds with heavy levels of exudate. Since highly exuding wounds are easily contaminated by bacteria, in recent years, there have been many attempts to develop dressings with antimicrobial properties.[23] Silver has been used as the antimicrobial component in a number of high-tech wound dressings.

Table III shows the release of silver ions from silver-containing chitosan fibers. It can be seen that when placed in normal saline 40 times its own weight, the silver concentration in the contacting solution reached 1.05 ppm after 24 h, while after 48 h, the silver concentration was 2.81 ppm. Literature information has shown that silver ion has effective antimicrobial properties at a concentration of 1 ppm or less.[24–26]

From the results shown in Tables II and III, it can be seen that chitosan fibers are a good carrier for silver ions. During the manufacturing process, silver ions can be easily attached to the chitosan fibers by treating the fibers with aqueous solutions of silver nitrate. When made into wound dressings, the silver ions can slowly migrate into the contacting solution, acting as an antimicrobial agent.

Figure 1 shows the zone of inhibition when chitosan fibers with and without silver ions were placed in contact with *E. coli* colonized plates. It can be seen that the silver-containing chitosan fibers have a much larger zone of inhibition than the original chitosan fibers, indicating the effectiveness of the silver ions as an antimicrobial agent. In Figure 2, it can be seen that the solution around the chitosan fiber and the silver-containing chitosan fibers were clear as compared to the turbidity in the control sample and in the viscose rayon sample, which demonstrated the antimicrobial properties of the chitosan and silver-containing chitosan fibers.

In Table IV, it can be seen that the silver-containing chitosan fibers are highly effective against *E. coli, S. aureus*, and *B. sabtilis*, with the bacteria concentration

TABLE IV
Antimicrobial Properties of Viscose Rayon, Chitosan, and Silver-Containing Chitosan Fibers

Type and number of bacteria	Control before shaking	After shaking			
		Control	Viscose rayon fiber	Chitosan fiber	Silver-containing chitosan fiber
Escherichia coli					
Number (CFU/mL)	1.0×10^4	1.4×10^7	1.6×10^7	3.2×10^6	20
Reduction (%)			−0.14	77.5	100
Staphylococcus aureus					
Number (CFU/mL)	2.0×10^3	1.6×10^5	1.5×10^5	2.8×10^4	4.2×10^3
Reduction (%)			0.06	82.5	97.4
Bacillus subtilis					
Number (CFU/mL)	1.0×10^3	1.0×10^5	1.0×10^5	2.2×10^4	7.0×10^2
Reduction (%)			0	78.0	99.3

reducing 100%, 97.4%, and 99.3% respectively, for these three types of bacteria after shaking for 8 h. Under the same test conditions, the chitosan fibers also showed some antimicrobial properties, with the reduction in the number of bacteria at 77.5%, 82.5%, and 78.0% respectively, for *E. coli*, *S. aureus* and *B. sabtilis*.

While silver ion is an effective antimicrobial agent, zinc ion is also important in wound healing.[21] There are ~200 zinc requiring enzymes in the human body, such as DNA polymerase which is needed for cell proliferation during healing. Zinc deficiency can lead to delayed closure of wounds and ulcers, while the collagen produced has reduced tensile strength. Zinc deficiency also affects the immune system by causing a reduction in lymphocytes, which leads to an increased susceptibility to recurring infection and poor wound healing. Clinical evidence has shown that topical application of zinc oxide inhibits bacterial growth for extended periods, especially of gram-positive bacteria. Topical administration of zinc chloride as a spray or ointment reduces the size of the wound, shortens healing time and produces less dehiscence.[21]

Chitosan fibers can be used as a carrier for zinc ions. Table V shows the effect of $ZnCl_2$ concentration on the zinc content in chitosan fibers when the fibers were placed in contact with aqueous $ZnCl_2$ solutions. Since previous studies have shown that the chitosan fibers can absorb a significant amount of zinc ions,[11] to attach a small amount of zinc ions on to the chitosan fibers for medical applications, relatively small amount of $ZnCl_2$ were used to treat the chitosan fibers. Results in Table V have shown that zinc ions can be easily attached to the chitosan fibers after treating the fibers with aqueous $ZnCl_2$ solution.

Table VI shows the effect of treatment time on fiber zinc content. Compared to silver ions, the absorption of zinc ions by chitosan fibers is relatively a fast process. Under otherwise same treatment conditions, the zinc content in the fibers remained unchanged after 5 h of treatment. While the equilibrium zinc content was 48.0 ppm, it was 12.6 ppm after 15 min of treatment. Twenty-six percent of the uptake was completed within the first 15 min.

Table VII shows the effect of time on the release of zinc ions from zinc-containing chitosan fibers. The zinc content in the contacting normal saline solution reached an equilibrium after 1 h of contact, and a significant amount of zinc ions were present in the solution, indicating the effectiveness of the zinc-containing chitosan fibers as a carrier for zinc ions.

CONCLUSIONS

This study has shown that when chitosan fibers are treated with aqueous solutions of silver nitrate and zinc chloride, silver and zinc ions can be attached to the fiber. When placed in contact with normal saline,

TABLE V
Effect of $ZnCl_2$ Concentration on the Zinc Content in Chitosan Fibers

	$ZnCl_2$ concentration (g/L)				
	0	6	7	8	8.5
Fiber zinc content (milligrams of zinc per gram of fiber)	1.58	16.28	19.02	19.79	26.19

TABLE VI
Effect of Treatment Time on Fiber Zinc Content

	Treatment time (h)				
	0.25	1	3	5	24
Fiber zinc content (milligrams of zinc per gram of fiber)	6.02	10.66	15.34	22.94	22.75

TABLE VII
Release of Zinc Ions from Zinc-Containing Chitosan Fiber

	Contact time (h)				
	1	3	5	8	24
Zn^{2+} concentration in contacting solution (ppm)	92.1	98.0	98.2	104.0	92.3

the silver and zinc ions can be released into the solution. Results have shown that the silver and zinc contents in the chitosan fibers can be controlled by controlling the concentration of the treatment solution and by controlling the treatment time. The silver-containing chitosan fibers are highly antimicrobial while the zinc-containing chitosan fibers can be used to administrate zinc to zinc deficient wounds.

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