Coated Cotton Gauze with Ag/ZnO/chitosan Nanocomposite as a Modern Wound Dressing

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ABSTRACT
Cotton gauze is one of the most successful wound dressings which utilize the intrinsic properties of cotton fibers. Modern wound dressings, however, require other properties such as antibacterial and moisture maintaining capabilities. In this study, conventional cotton gauze was treated with chitosan/Ag/ZnO nanocomposite for achieving modern wound dressing properties. Cotton gauze samples were impregnated with chitosan/Ag/ZnO nanocomposite by the dip, dry, and cure method. Samples were characterized by scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FTIR) and UV-Vis reflective spectroscopy (UV-Vis); and their water absorbency ability, wicking effect, and antibacterial activity determined. The results show that coated cotton gauze with chitosan/Ag/ZnO nanocomposite has increased drying time (78%) and water absorbency (38%). Furthermore, their antibacterial efficiency was 96% for Escherichia coli (E. Coli) and 99% for Staphylococcus aureus (S. aureus).

Keywords: Cotton Gauze; wound Dress, Chitosan, Ag, ZnO, Nanocomposite

INTRODUCTION
In recent years, wound dressing has evolved according to the pathogenesis of different wounds. Different types of wound dressing including xerogels, charcoal cloth, alginates, chitosan, and hydrogels have been introduced for this purpose through the last decades [1]. An ideal wound dressing has the ability to maintain moisture, act against microorganisms, be nontoxic, non-adherent, and promote wound healing [2]. Modern wound dressing theory, suggests promoting dynamic equilibrium between exudate absorption and optimal surface moisture at the wound surface. In addition, it should be able to exchange gas to provide the wound with sufficient oxygen tension. Combining new materials, better design and complex manufacturing are needed for achieving these goals [3].

Recently, many researchers have focused on the use of biological materials like chitin and chitosan [4-6]. Chitosan as a biomaterial, has a great potential in wound healing and skin burns and regenerates normal skin [7, 8]. It is an antibacterial, nontoxic, biodegradable, and biocompatible polymer [9-11]. Chitosan, along with other antibacterial materials has attracted much attention during the last few years. For instance, its combination with other inorganic agents such as Ag, Zn, CuO, TiO2 and Fe has been reported [12-16]. Among them, chitosan/Ag/ ZnO nanocomposite had significantly higher antibacterial activity than chitosan/Ag and chitosan/ZnO blend films. It was indicated that Ag and ZnO enhanced the antibacterial activities of chitosan [14].

The anti-bacterial and multi-functional properties of silver nanoparticles have also been investigated [17, 18]. Products containing silver nanoparticles have the ability to exert bactericidal effects and are less harmful to human cells than other toxic organic antimicrobial agents [12, 19]. Tiam et al [20] found that Ag nanoparticles not only have antibacterial activity, but also have wound healing properties. It can restore burnt skin to the normal skin.

Zinc is normally required for a variety of enzymatic and cellular activities. But because of its excellent anti-inflammatory, drying, mild astringent and antiseptic properties, it plays a major role in wound healing, especially from burns. It also supports the demand for volume increases during the wound healing process. For that purpose, Zinc oxide is widely used in skin creams. This cream can prevent sunlight and ultraviolet rays from penetrating the skin

and can enhance the wound healing process by delivering zinc ions to the wound and allowing them to remain there for an extended period of time [21]. Although proposed, the theory is that zinc accelerates re-epithelialization of the skin; the exact mechanism is really not known [22-24].

In this study, conventional cotton gauze was coated with a nano emulsion of chitosan/Ag/ZnO to benefit from the supreme properties of Ag, ZnO nanoparticles and chitosan, such as prevention of embedded nanoparticles agglomerations, beside the intrinsic properties of cotton fibers. Some essential factors of modern wound dressings like water absorbency, drying time (water holding time) and the amount of vertical wicking were determined for different treated samples and were compared with untreated samples.

EXPERIMENTAL

Materials

High molecular weight chitosan (DS=75%), silver nanoparticles (average size of <150 nm) were purchased from Sigma Aldrich Co., Ltd (USA). Zinc oxide nanoparticles, (average particle size ≈ 20 nm) were provided by Nano Pars Lima Co., Ltd. (Iran). Escherichia coli (ATCC 6538) and Staphylococcus aureus (ATCC 4157) were prepared by Microorganism Lab of Azad University.

Preparation

Chitosan was dissolved at 0.5% (w/v) with 1% (v/v) acetic acid and then 5 ml (5% w/v) Ag and 5 ml (5% w/v) ZnO nanoparticles were added to the prepared solution. The cotton gauze was dipped in the CS/Ag/ZnO colloidal solution; dried at 80 °C and cured at 160 °C for 4 min.

Characterization

The presence of ZnO nanoparticles was proved by a UV-Vis spectrophotometer (JASCO-ARN-475, UK) due to their reflective properties and FTIR spectrum (Thermonicolet-Nexus 870, USA) with considering their characteristic peaks. The morphology of the treated cotton gauze was studied using a Philips XL30 scanning electron microscope (SEM) equipped with Philips-EDAX/DX4 energy-dispersive Spectroscope (Netherlands).

AATCC-147-1998:

Qualitative Antibacterial-Agar Diffusion Test:

Antibacterial proficiency of samples determined qualitatively (AATCC-147-1998) against a gram positive organism (*Staphylococcus aureus*) and a gram negative bacterium (*Escherichia coli*). In qualitative antibacterial-agar diffusion test, samples (ϕ=4.8±0.1 cm) were incubated at 120 °C for 15 min. Bacterium was placed in 5 ml nutrient broth and incubated at 37 °C for 24 hrs. Agar Petri dishes were inoculated with grown bacterium. Treated (with chitosan, chitosan/Ag, chitosan/ZnO and chitosan/Ag/ZnO) and untreated samples were gently pressed in the centre of media culture. The plates were incubated at 37°C for 24 hours and zone of inhibition observed [25, 26].

AATCC-100-1998:

Quantitative Test-Percentage Reduction Test

In quantitative (AATCC-100-1998) test method samples (ϕ=4.8±0.1 cm) were incubated at 120°C for 15 min [27]. The bacterial culture was grown using the same method as mentioned. The sterile samples were placed in a 250 ml glass jar and the samples were dipped in 1ml bacteria with 1000cfu/ml concentration and were incubated at 37°C for 24 hours. 100 ml of sterilized distilled water was added into the jar and then shacked continuously for one min. 1 ml of solution was diluted and was placed to in 25 ml of nutrient agar and incubated at 37°C for 24 hours. Colonies of bacteria, recovered on the agar plate, were counted and the reduction percentage of bacteria (R) was calculated by the Eq. (1):

$$R\ (\%) = (B - A) \times 100 / B \qquad (1)$$

Where A is the number of bacterial colonies from treated specimen after inoculation over 24 hrs contact period, and B is the number of bacterial colonies from untreated control specimen after inoculation.

Vertical Wicking Test

For measuring water transport rate, the vertical wicking test was used [28]. Treated samples with chitosan/Ag/ZnO were cut (170 mm by 25 mm). One end of strip was immersed about 3 mm in water. The height of water transported along the strip in different times (1, 5 and 10 min) was then measured. Vertical wicking test was evaluated 5 times and the results were reported by mean values.

Water Holding Time

A test method offered [28] by T-PACC (centre for research on textile protection and comfort) in North Carolina State University (NSCU) was used for measuring drying time of samples. At first, circular cuts (ϕ=3.5 inch) of samples were prepared and weighed. Then gauzes were wetted with 1^{cc} distilled water and weighted. The reduced weight of samples was measured every 15 min until the measured weight equalled to primary weight.

Water Absorption Test

The water absorption was determined according to static immersion test (BS 34491 (1990) standard) for fabric with high ability of water absorption [29]. The water absorption percentage (W) was calculated by Eq. (2):

$$W= (M_2-M_1)/M_1 \quad (2)$$

where M2 is the weight of wet sample, and M1 is the weight of dry sample.

RESULTS AND DISCUSION

Figure 1 demonstrated the presence of ZnO on cotton samples. According to Rayleigh's scattering theory, presence of nanoparticles with the particle size between 20 and 40 nm causes wide distribution around UV region [30].

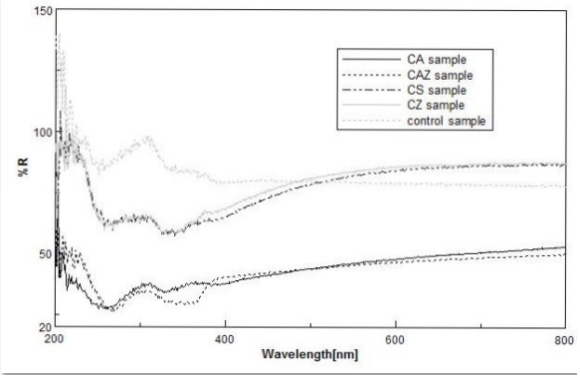

FIGURE 1. UV–vis spectra of the samples: the treated sample with chitosan/Ag/ZnO (CAZ), the treated sample with chitosan/Ag (CA), the treated sample with chitosan/ZnO (CZ), the treated sample with chitosan and untreated sample.

Figure 2 presents SEM photographs of treated sample with chitosan/Ag/ZnO and untreated sample and chitosan/Ag/ZnO nanocomposite. From SEM images the formed coating appears homogeneously on the cotton gauze surface.

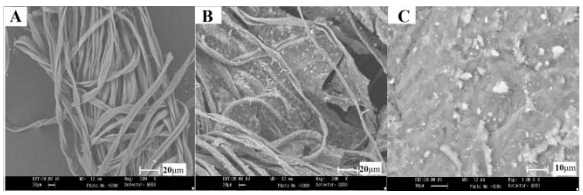

FIGURE 2. SEM photographs of the sample: (a) untreated sample (b) the treated sample with chitosan/Ag/ ZnO (c) chitosan/Ag/ ZnO nanocomposite.

Figure 3 illustrates the EDX spectrum of chitosan/Ag/ZnO nanocomposite. As shown in *Figure 3,* ZnO and Ag elements were identified. This analysis verified that chitosan/Ag/ZnO nanocomposite was formed. The result agreed with the SEM as well (*Figure 2b*).

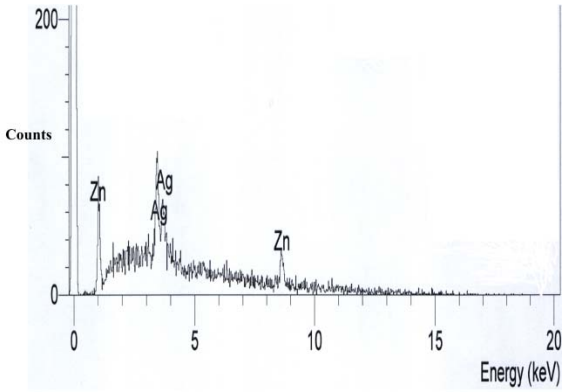

FIGURE 3. EDX spectrum of chitosan/Ag/ZnO nanocomposite.

Figure 4a and *Figure 4b* depict the FTIR of chitosan and chitosan/Ag/ZnO nanocomposite, respectively. Chitosan FTIR shows absorption peak at 3447 cm^{-1}, this attributed to the combined peaks of NH_2 and OH group stretching vibration [31]. Compared with chitosan, the broader and stronger peak moved to lower wave number at 3390 cm^{-1} which indicated the interaction between this group and ZnO [32]. The absorption peak at 2995 is attributed to asymmetric stretching oH, CH_3 and CH_2 of chitosan polymer. The absorption peaks of 1637 and 1066 belong to bending vibration of NH_2 group and C-O stretching group. Compared with chitosan, there is new absorption peak at 649 which belong to attachment of amide group and stretching of ZnO [32]. Addition of silver nanoparticles shifted the characteristic peak of amide 1637 from 1652 are ascribed to incorporate of silver nanoparticles into the nanocomposite [33, 34].

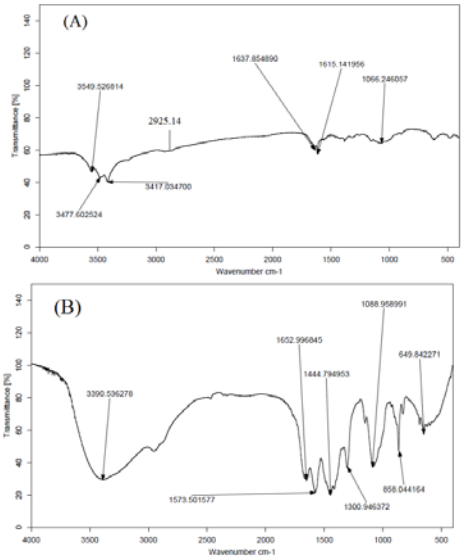

FIGURE 4. FT-IR spectrum (a) chitosan and (b) chitosan/Ag/ZnO nanocomposite.

Antibacterial Activity

The overall view of antibacterial test against S. aureus and E. Coli is demonstrated in *Figure 5*.

The qualitative antibacterial assessments of samples are shown in *Figure 6* and *Figure 7*. As shown in these figures, the sample treated with chitosan/Ag/ZnO nanocomposite showed better antibacterial effects against S. aureus and E. Coli. Usually antibacterial activity of treated sample against S.aureus as a gram positive bacterium is better than E.coli as a gram negative bacterium.

The quantitative antibacterial assessments of samples are shown in *Figure 8* and *Figure 9*. As shown in these figures the sample treated with chitosan/Ag/ZnO nanocomposite showed better antibacterial effect against S. aureus and E. Coli.

The results of quantitative antibacterial tests (*Table I*) show that the treated sterile gauze with chitosan/Ag/ZnO nanocomposite has a 99% reduction for S. aureus and a 96% reduction for E.coli. The other treated samples revealed a lower percentage of reduction against S. aureus.

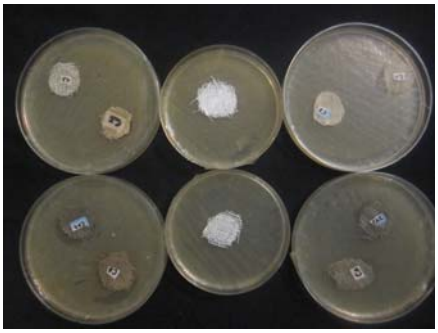

FIGURE 5. Overall view of antibacterial test against S. aureus and E. Coli.

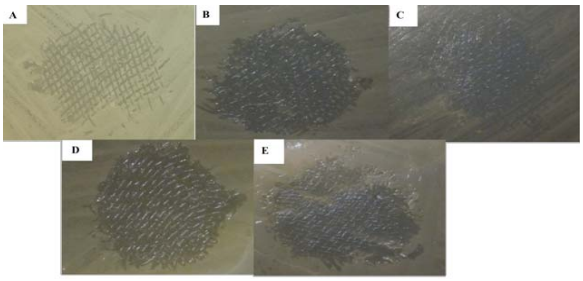

FIGURE 6. Zones of inhibition for samples against E. coli: (a) untreated (b) treated with chitosan (c) treated with chitosan/Ag (d) treated with chitosan/ZnO (e) treated with chitosan/Ag/ZnO.

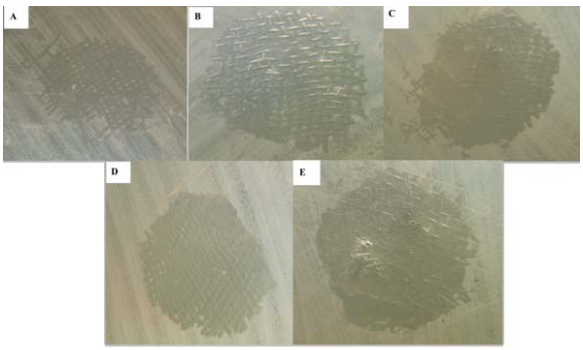

FIGURE 7. Zones of inhibition for samples against S. aureus: (a) untreated (b) treated with chitosan (c) treated with chitosan/Ag (d) treated with chitosan/ZnO (e) treated with chitosan/Ag/ZnO.

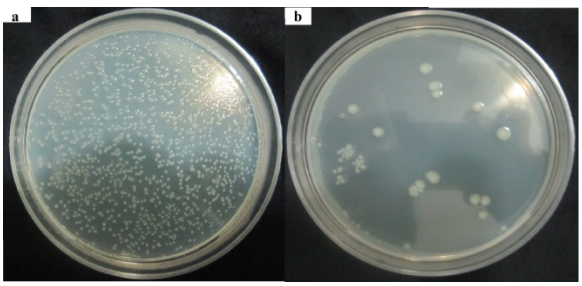

FIGURE 8. Quantitative (ATCC-100) test against E. Coli (a) untreated (b) treated with chitosan/Ag/ZnO.

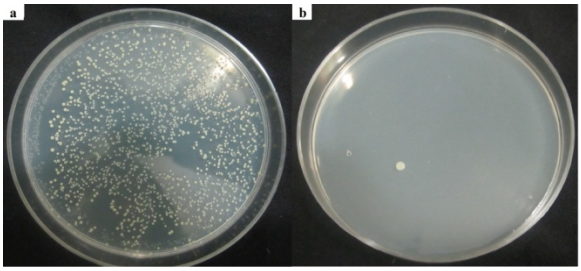

FIGURE 9. Quantitative (ATCC-100) test against S. aureus (a) untreated (b) treated with chitosan/Ag/ZnO.

TABLE I. Number of colonies and their reduction percentage (R%) on samples.

Sample Type	Number of S.aureus colonies	R (%)	Number of E.coli colonies	R (%)
Untreated	1000 >	0	1000 >	0
Treated with chitosan	500	50	700	30
Treated with chitosan/ZnO	30	97	300	70
Treated with chitosan/Ag	2	99	250	75
Treated with chitosan/Ag/ZnO	1	99	40	96

Vertical Wicking Test

The vertical wicking ability of treated sample with chitosan/Ag/ZnO and untreated one were measured three different times. Each time, the test was repeated five times and the mean value reported in *Table II*. The results show that the wicking ability of treated gauze and untreated gauze both increased though time. The results of "ANoVA" show P_{value}= 0.01 which is smaller than the significant 0.5 level. So difference between treated cotton gauze and untreated one is statistically significant (95%).

TABLE II. Vertical wicking ability of untreated and treated gauzes in different times.

Time (min)	Untreated gauze	Treated gauze with chitosan/Ag/ZnO[a]
1	1.63	2.67
5	3.26	5.16
10	4.76	6.02

[a] Each test was repeated five times and for every time the mean was reported

Water Holding Time

Table III shows average drying time for each sample. For this, the differences between untreated cotton gauze and the treated ones were measured. The data set from "ANOVA" shows that the P_{value}= 0, so the null hypothesis is refused and there is a statistically significant difference between untreated cotton gauze and treated one is at level of 95%. The average water holding ability of treated gauze is 78 percent higher than untreated ones. Holding capability of liquid especially water is a fundamental factor for healing the wounds [28].

TABLE III. Average drying times (water holding ability) of wet samples.

Sample type	Mean time of drying (min)[a]
Untreated	41.31
Treated with chitosan/Ag/ZnO	73.60

[a] Each test was repeated five times and the mean reported

Water Absorption Test

The water absorption percentages of samples are shown in *Table IV*. The difference between untreated cotton gauze and treated one is statistically significant (P_{value}=0). Comparing data shows that capability in water absorption of cotton gauze had increased about 38 percent.

When the spaces of cotton gauze between wraps and wefts are filled with chitosan, due to channel-like structure of chitosan [35], the number of capillaries of cotton gauze increased in both weft and wrap directions. Therefore, vertical wicking and water absorption increased. This ability is highlighted for water, wound exudates, and liquid drug absorption of a wound dressing [29].

TABLE IV. Water absorption (static immersion test) of samples.

Sample Type	Mean percent of water absorption (%)[a]
Untreated	293
Treated with chitosan/Ag/ZnO	407

[a] Each test was repeated five times and the mean reported.

CONCLUSION

The present study shows that treating conventional cotton gauze with a nanocomposite of chitosan/Ag/ZnO will improve its wound care ability toward modern wound dressings. Chitosan, as one of the natural polysaccharides, is a nontoxic, biodegradable polymer with proper biological activities. It showed high potential for holding nanoparticles and also for coating cotton gauze. The emulsion of such nanocomposite can be easily applied onto textile fabrics using a simple method like the dip-dry-cure process. Antibacterial efficiency values of treated cotton gauze with chitosan/Ag/ZnO were increased in comparison with each of the nanoparticles separately indicating a kind of synergetic effect for a nanocomposite of chitosan/Ag/ZnO. Treating cotton gauze with

chitosan/Ag/ZnO nanocomposite also increased drying time, wicking ability, and water absorbency; the main indexes of a modern wound dressing.

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