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Novel Atmospheric Plasma Enhanced Chitosan Nanofiber/Gauze Composite Wound Dressings

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ABSTRACT: Electrospun chitosan nanofibers were deposited onto atmospheric plasma treated cotton gauze to create a novel composite bandage with higher adhesion, better handling properties, enhanced bioactivity, and moisture management. Plasma treatment of the gauze substrate was performed to improve the durability of the nanofiber/gauze interface. The chitosan nanofibers were electrospun at 3–7% concentration in trifluoroacetic acid. The composite bandages were analyzed using peel, gelbo flex, antimicrobial assay, moisture vapor transmission rate, X-ray photoelectron spectroscopy (XPS), absorbency, and air permeability tests. The peel test showed that plasma treatment of the substrate increased the adhesion between nanofiber layers and gauze substrate by up to four times. Atmospheric plasma pretreatment of the gauze fabric prior to electrospinning significantly reduced degradation of the nanofiber layer due to repetitive flexing. The chitosan nanofiber layer contributes significantly to the antimicrobial properties of the bandage. Air permeability and moisture vapor transport were reduced due to the presence of a nanofiber layer upon the substrate. XPS of the plasma treated cotton substrate showed formation of active sites on the surface, decrease in carbon content, and increase in oxygen content as compared to the untreated gauze. Deposition of chitosan nanofibers also increased the absorbency of gauze substrate.
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INTRODUCTION

Traditional textile-based wound dressings fail to provide optimal wound healing conditions (hemostasis, non-adherence, maintenance of a moist wound bed, etc.).[1,2] Recently, many advanced wound dressings have been developed to provide enhanced functionalities and help to maintain an appropriate healing environment around wounds, but also led to higher cost and difficulty in handling.[3,4] The goal of this research is to create chitosan nanofiber/gauze composite bandages that combine the desirable properties of each component, and to characterize the physical and mechanical properties of these materials in order to predict the performance of these novel materials for wound care applications.

Among various wound dressing materials, chitosan has been extensively investigated due to its inherent biocompatibility, biodegradability, antimicrobial activity, wound healing property, and antitumor effect.[5] Chitosan has previously been used for other related biomedical applications, including drug delivery and bone healing.[6–8] Aoyagi et al.[9] formulated wound dressing films made of chitosan, minocycline hydrochloride, and Tegaderm™ as backing materials for the treatment of severe burns. Wound protection and controlled drug release were achieved

using these chitosan containing films. Studies on sponge-like asymmetric chitosan membranes by Mi et al.[10] demonstrated the oxygen permeability, as well as hemostatic and antibacterial properties of chitosan.

In the context of wound dressings, nanofibers have been shown to have functional versatility, including desirable wound adherence, absorption, oxygen permeability, resorbability, and occlusivity.[11–13] Their high specific surfaces make them superlative matrices for controlled drug release. Nanofiber webs form highly effective filters for the contaminants, particulates, and microorganisms without sacrificing air and moisture permeability.[11–13]

Owing to the antibacterial properties of chitosan and the advantages of electrospun nanofibers, several attempts were made by researchers to electrospin chitosan nanofiber webs. However, electrospinning of pure chitosan was problematic due to its highly crystalline nature and inability to dissolve in solvents; instead, many previous studies relied on blends of chitosan/poly(vinyl alcohol) (PVA)[14] and chitosan derivatives.[15] Subsequently, successful electrospinning of pure chitosan in trifluoroacetic acid (TFA) was reported by Ohkawa et al.[16] They derived a linear relationship between nanofiber diameter and electrospinning solution concentration. In other work, electrospinning of pure chitosan was carried out in aqueous 90% acetic acid at 7% concentration.[17] However, characterization of mechanical strength, antibacterial properties, and absorbency of chitosan nanofibers was not reported.

Nanofiber webs are inherently weak and difficult to handle.[18,19] Deposition of nanofiber coatings onto conventional textile bandages addresses the need for structural support,[19] but the challenge of delamination due to compliance mismatch or poor adhesion remains. While numerous studies of chitosan-based nanofibers as components of wound dressings have shown promise, properties of pure chitosan nanofibers in wound dressings have not yet been completely characterized. Additionally, the adhesion of primary wound dressing layer of chitosan nanofibers electrospun onto a secondary cotton wound dressing substrate layer is a critical issue that has not been fully addressed in published work.

In our previous studies, atmospheric pressure plasma technology has been used to modify the structures and properties of various materials (especially textile materials). We have shown that plasma treatment can: (i) improve surface bonding or adhesive ability,[20,21] (ii) increase mechanical strength by crosslinking,[20–24] (iii) change fiber surface hydrophobicity,[25] (iv) roughen fiber surfaces,[26] (v) increase crystallinity.[26,27] Recently, Vitchuli et al.[18] successfully employed atmospheric plasma treatment to improve the adhesion between nylon nanofibers and nylon/cotton fabric substrates.

In this study, pure chitosan nanofiber webs were electrospun onto plasma-treated 100% cotton (gauze) substrates to produce mechanically strong, highly absorbent, antibacterial, biocompatible, composite wound dressings. The composite materials were characterized to determine:

- Effects of plasma pretreatment of the substrate on the bonding between nanofiber mats and textile substrates,
- Absorbency,
- Air permeability
- Moisture vapor transport rate
- Antimicrobial activity.

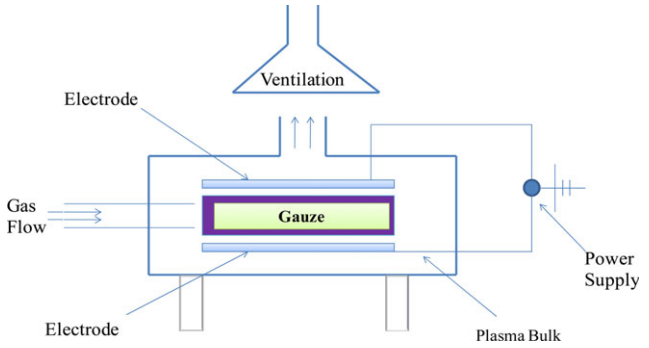

Figure 1. Schematic diagram of atmospheric pressure plasma system. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

MATERIALS AND METHODS

Materials

Chitosan (Sigma Aldrich, molecular weight: 190 kDa–375 kDa, degree of deacetylation: 85%) was used for electrospinning onto a 100% cotton gauze substrate (Carolina Narrow Fabric Co. TFA (Sigma Aldrich) was selected as the solvent.

Electrospinning of Nanofiber Webs Onto Substrates

The electrospinning equipment consisted of an extrusion system (syringe pump), fiber collection system (rotating drum), and high voltage power supply. The syringe was fixed onto the extrusion system and was filled with chitosan solution to produce the desired spinning yield. A positive charge was applied to polymer solution via high voltage power supply. Chitosan nanofibers were electrospun in the range of 2–7% concentration in TFA with an extrusion rate of 1.5 mL/h for 2 h, applied voltage of 25 Kv, and distance of 15 cm in between rotating drum and needle tip. The collection system for nanofibers includes a cylindrical polyvinyl chloride (PVC) drum with a grounded metal ring at one end. A metal rod is attached to the ring and runs along the length of the cylinder. In order to make the surface conductive, aluminum foil was wound over the drum in contact with the metal rod. The textile substrate was then wrapped around the aluminum foil to enable deposition of electrospun nanofibers onto it. The speed of the drum was maintained at 20 rpm using a motor.

Plasma Pretreatment of Substrate

The atmospheric pressure audio frequency glow discharge system was designed and developed at North Carolina State University. The capacitively-coupled dielectric-barrier-discharge (DBD) consists of two parallel copper electrodes, each embedded within a Lexan polycarbonate insulator as shown in Figure 1. Stable and uniform plasma was achieved at a low (audible) frequency of 1.373 kHz during the operation. The voltage across the plates was ~6.3 kV_{rms} and 7.6 kV_{max} for 100% He plasma and ~6.6 kV_{rms} and 7.85 kV_{max} for 99% He plus 1% O_2. Gas flow rates were 20 L/min for helium gas and 0.3 L/min for oxygen gas.

The gauze substrates were placed on a nylon grid suspended in the middle of the plasma chamber to enable complete and

uniform plasma exposure from all sides. The fabrics were treated with 100% He-plasma or 99%He/1%O_2 plasma prior to nanofiber deposition. After nanofiber deposition, the samples were conditioned at standard temperature of (20 ± 1)°C and relative humidity of (65 ± 2)% for at least 8 h before they were tested for adhesion, antibacterial properties, moisture vapor transmission rate, air permeability, and surface chemical composition (via X-ray photoelectron microscopy).

Adhesion Between Nanofiber Mats and Supporting Fabrics—Peel Test

A peel test method was devised for evaluating the adhesion between the nanofiber mat and the fabric substrate based on ASTM D2261—Tearing strength of woven fabrics by tongue (Single Rip) method[28] using an Instron Tensile Tester. The 90° peel test was used. This method allows the user to control the rate of delamination and the locus of failure. Substrate samples (5 cm × 1 cm) with chitosan nanofibers deposited on them were tested. Pieces of masking tape were used to couple the two layers, i.e., substrate (fabric) and nanofiber mat, to the lower and upper jaws, respectively. The speed of the upper jaw was kept constant at 50 mm/min. A 50 g load cell with a gauge length of 1.27 cm was used to measure the force of adhesion. For each sample, 6–8 specimens were tested on an Instron® Tensile Tester and the average force to peel off the nanofiber layer off the substrate was recorded.

Gelbo Flex Tester

In order to assess the ability of nanofiber mats electrospun onto the substrate to withstand repetitive strain, Gelbo Flex testing was employed using a modification of ASTM F 392-93, Standard Test Method for Flex Durability of Flexible Barrier Materials.[29] Nanofiber-deposited fabric samples of size 5 cm × 1 cm were attached to two circular clamping disks, via hose clamps, and the samples were twisted and flexed for 1000 cycles. Results of Gelbo Flex testing provided a visual adhesion assessment which was observed under scanning electron microscopy (JEOL 6400 F Field Emission SEM).

Antibacterial Tests

Antibacterial testing was performed on plasma treated cotton gauze with and without chitosan nanofibers. Cultures used for the antibacterial tests were *Escherichia coli* O157 strain B179 and *Bacillus cereus* strain B2, obtained from the USDA/ARS Food Science Research Unit culture collection, Raleigh, NC. A 5 mL of broth culture was prepared for each organism, *E. coli* in Lauria Broth (LB broth, BD Diagnostic Systems, Sparks, MD) at 37°C, and *B. cereus* in Tryptic Soy Broth (TSB, BD Diagnostic Systems) at 30°C, with shaking for aeration at 200 rpm in a 15-mL centrifuge tube (Corning®). In a biological safety cabinet, pieces of fabric ~5 mm square were aseptically cut and placed in 1.5 mL tubes. Broth cultures were harvested at 5000 rpm for 10 min, and resuspended in an equal volume of saline to give approximately 10^9 CFU/mL. An aliquot (200 μL) of each cell suspension was added to the tubes with fabric: *B. cereus* with chitosan-free fabric, *B. cereus* with chitosan-treated fabric, *E. coli* with chitosan-free fabric, and *E. coli* with chitosan-treated fabric. After incubation of *E. coli* at 37°C and *B. cereus* at 30°C with shaking at 200–300 rpm, the surviving cells were appropriately diluted (three dilutions of the cell suspensions were subsequently used: 0, 10^3, and 10^5 times).and plated on LB (*E. coli*), or TSB (*B. cereus*) using a spiral plater (Autoplate 4000®, Spiral Biotech, Norwood, MA). Plates were incubated for 24 h at 37°C for *E. coli* and 30°C for control and *B. cereus*, and the colonies were counted using an automated plate reader (Qcount®, Spiral Biotech).

Air Permeability

The air permeability of electrospun fiber-deposited fabrics was measured using a Frazier air permeability testing instrument (Frazier Precision Instrument Co., MD). The measurement was carried out according to ASTM D737-04 Standard Test Method for Air Permeability of Textile Fabrics,[30] with 1 mm and 1.4 mm orifices for composite bandages and an 8 mm orifice for cotton gauze (since it has a more open structure than composite bandages), 6.45 cm^2 test area, at 760 mm mercury pressure, 21°C, and 65% RH. An average of 10 readings of air permeability from different parts of the composite bandages was recorded.

Moisture Vapor Transmission Rate

Measurements for moisture vapor transmission rate (MVTR) of the samples (100% gauze with and without chitosan nanofibers) were made in at the standard atmosphere laboratory temperature and humidity (21°C and 65% RH). The rate of moisture vapor diffusion through the material was determined according to the Simple Dish Method similar to ASTM E96-80, Standard Test Method for Water Vapor Transmission of Materials.[31] A sample was placed on a water dish (82 mm in diameter and 19 mm in depth) with a 9 mm air space between the water surface and specimen. A vibration free turntable with eight dishes, rotating at a uniform speed of 5 m/min was used to ensure that all dishes were exposed to the same average ambient conditions during the test. The specimen dishes were allowed to stabilize for 2 h before taking the initial weight. The final weight was measured after a 24-h interval. The MVTR was calculated in units of g/m^2-24 h. The experiment was repeated five times for each sample.

XPS Analysis

X-ray photoelectron spectroscopy (XPS) was carried out to investigate the changes in the surface chemical composition of 100% cotton gauze substrate as a result of changes in plasma parameters (percentage of Helium and oxygen). Spectra were obtained using a Riber LAS-3000 with MgKα excitation (1254 eV). Energy calibration was established by referencing to adventitious Carbon (C1s line at 284.5 eV binding energy). A takeoff angle of ~75° from surface was used with an X-ray incidence angle of ~20° and an X-ray source to analyzer angle of ~55°. Base pressure in the analysis chamber was in 10^{-10} Torr range. CASA XPS software was used for data reduction.

Absorbency

An absorbency test was carried out on the composite wound dressing and gauze fabric in accordance with BS EN 13726-1: 2002 Test methods for primary wound dressings—Part 1: Aspects of absorbency, Section 3.2—Free Swell Absorptive Capacity.[32] The composite dressing was cut into 4 cm × 4 cm pieces and was weighed (W_0). A solution consisting of 142 mmol Na^+ ions and 2.5 mmol Ca^{2+} ions was first pre-warmed to 37°C. The composite nanofiber dressing was placed in a Petri dish and treated with the above mentioned solution (weight of solution being at least 40 times the weight of sample) and incubated at 37°C for 30 min). After 30-min incubation the Petri dish was removed from the incubator, and was suspended for 30 sec on paper towel[33] before being reweighed (W_{30}) so as to allow the excessive solution to run off. The procedure was repeated five times. The

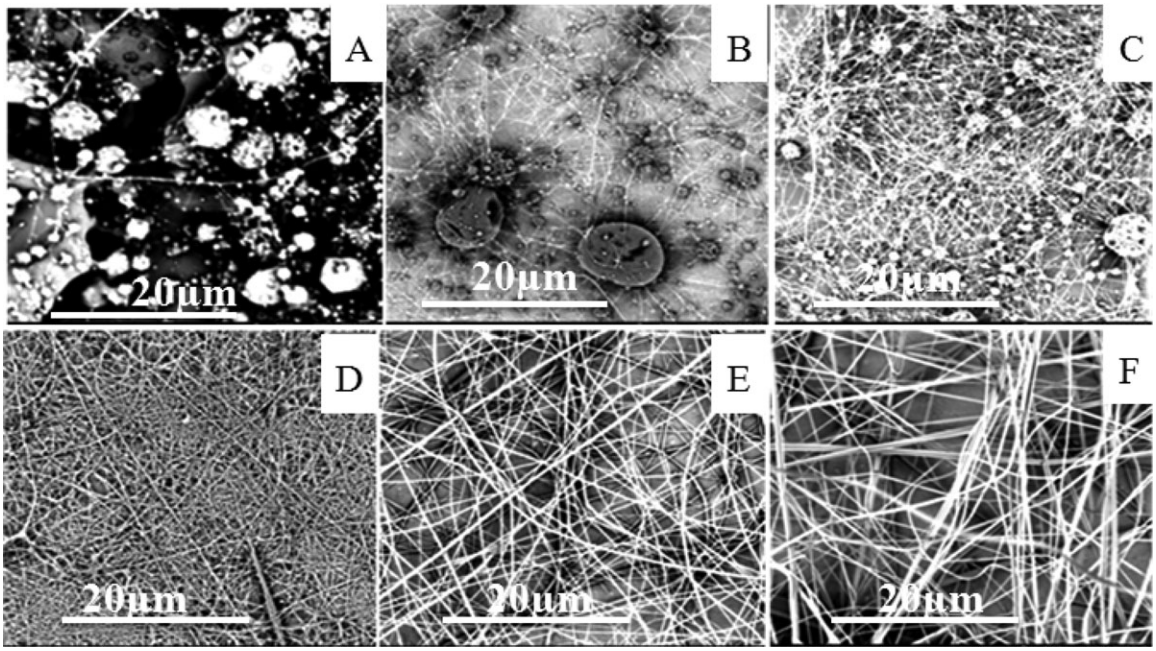

Figure 2. SEM images of chitosan nanofibers concentration: (A) 2%, (B) 3%, (C) 4%, (D) 5%, (E) 6%, and (F) 7% in TFA. Extrusion rate: 1.5 mL/h, applied voltage: 25 kV, distance: 15 cm.

absorbency of the dressing was expressed as amount of solution absorbed per square centimeter dressing ($[W_{30} - W_0]$/area). The absorbency can be expressed in terms of g/cm^2 ($[W_{30} - W_0]$/area) or percentage ($[W_{30} - W_0] \times 100/W_0$).

RESULTS AND DISCUSSION

Electrospinning of Chitosan Nanofibers

Chitosan nanofibers were electrospun in the range of 2–7% concentration in TFA (Figure 2). Electrospinning of 2% concentration of chitosan in TFA showed bead formation with no fiber formation. However, as the concentration increased from 3 to 7%, an obvious reduction in number of beads, with an increase in fiber forming tendency was observed. A 1% increment in concentration from 3 to 7% yielded increasing average diameters of 97, 105, 116, 165, and 252 nm, respectively. Concentrations above 7% are not considered because the increase in average diameter will significantly decrease the specific surface area and porosity of the nanofiber web. All further experiments were performed at 7% concentration.

Adhesion Between Nanofiber Mats and Supporting Fabrics

The peel test results showed that after treating the substrate with 100% helium plasma and 99% helium/1% oxygen plasma, the force required to peel off the nanofiber layer from substrate was increased by up to approximately four times (Table I). While control sample nanofiber webs were removed intact, tearing of the nanofiber web was observed (Figure 3) during the peel tests on plasma treated substrates (Figure 3), indicating improved adhesion between nanofiber layers and the substrates. Gelbo Flex testing was performed on the samples and their morphologies were observed in SEM at × 15 magnification as shown in Figure 4. It was observed that the nanofiber layer was badly damaged after 1000 cycles in the case of untreated substrate, whereas it showed much better durability when the substrate was pretreated by plasma. In the latter case, the nanofiber layer was intact and firmly adhered to the substrate as a whole layer.

Table I. Measurement of Adhesion Between Nanofiber Mats and Supporting Fabrics

Treatment on substrate	Control	He (Pre-treatment)	He + 1% O_2 (Pre-treatment)
Mean (gf)	11.05	42.28	31.23
Standard Deviation	1.95	6.12	1.24

Antibacterial Test

An open wound is highly susceptible to infectious bacteria.[34] Once the wound becomes infected, it requires additional treatments that are painful and result in delayed healing. The use of antimicrobial materials in wound dressings in a non-occlusive dressing provides enhanced protection while maintaining good moisture management properties. The addition of chitosan nanofibers to the substrate gauze significantly increases the antimicrobial activity of the bandage, and is expected to aid in infection prevention via contact with bacteria in or surrounding the wound.

Results of antimicrobial tests are shown in Table II. The control gauze substrate showed no antimicrobial activity, while the chitosan-gauze composites showed antimicrobial activity against both *E. coli* and *B. cereus*. It is clear from Table II that untreated gauze fabric with chitosan nanofibers showed log reduction of 4 as compared to that of untreated gauze for both *E. coli.* and *B. cereus*. The cationic amine group in chitosan is responsible for

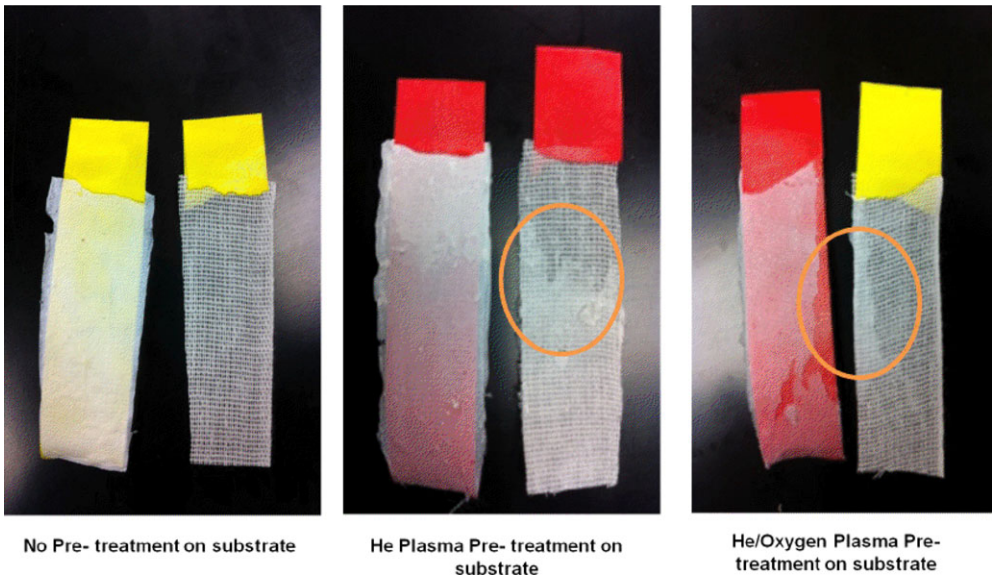

Figure 3. Evaluation of nanofiber–fabric adhesion using the 90 degree peel test. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

its antibacterial activity. Figure 5 and Table II show that the antibacterial activity of the chitosan nanofiber webs retained after treating the substrate with He- and He/O_2-plasma. While He-plasma pretreated gauze fabric with chitosan nanofibers showed 2.5 and 2.5 log reductions, respectively, He/O_2 plasma pretreated gauze fabric with chitosan nanofibers showed 2.5 and 3.5 log reduction for both *E. coli.* and *B. cereus*, respectively. From the above results, we can conclude that the plasma pretreatment did not change the antibacterial properties of the cotton gauze fabric, while the introduction of the chitosan nanofiber layer significantly improved the antibacterial properties (Figure 5 and Table II).

Air Permeability

Moisture management is critical to wound healing, and is governed by factors including air permeability, moisture vapor transmission, and fluid absorbency. While too much moisture in the bandage microclimate results in maceration, too little

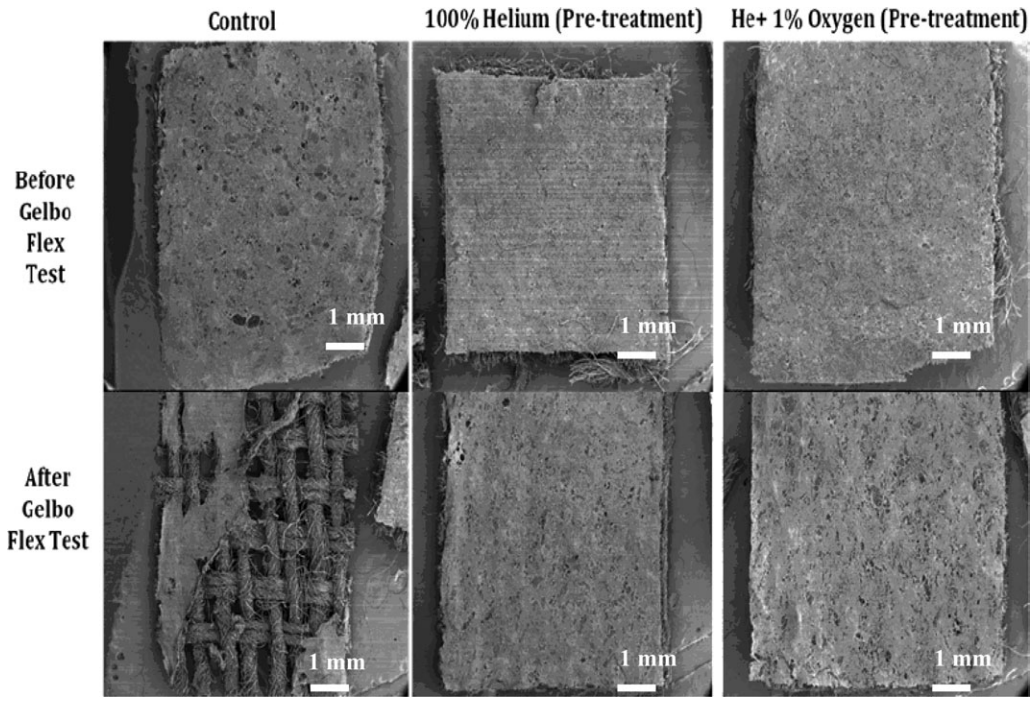

Figure 4. SEM images of composite bandages, before Gelbo testing and after 1000 cycles of Gelbo flex testing with no pretreatment, 100% He, 99%He/1%O_2 plasma pretreatment on substrate.

Table II. Antibacterial Activities of Chitosan Nanofiber Coated Gauze Fabric Against Only Gauze

Samples	*E. coli* [log(CFU/mL)]	*B. cereus* [log(CFU/mL)]
Only bacteria	7.52 ± 0.01	6.55 ± 0.08
Gauze	7.47 ± 0.09	6.14 ± 0.14
He treated gauze	7.01 ± 0.36	5.73 ± 0.25
He/O_2 treated gauze	7.51 ± 0.14	5.69 ± 0.03
Gauze + chitosan	3.65 ± 0.02	2.98 ± 0.1
He-treated gauze + chitosan	5.09 ± 0.53	4.18 ± 0.81
He/O_2 treated gauze + chitosan	4.82 ± 0.06	2.80 ± 0.06

Table III. Average Air Permeability and Moisture Vapor Transmission Rate (MVTR) of the Composite Wound Dressing, i.e. Chitosan Nanofiber Electrospun on Untreated, He Plasma Treated, and He-O_2 Plasma Treated Cotton Substrate

Samples	Air permeability ($cm^3/cm^2/sec$)	MVTR ($g/m^2/day$)
Gauze	265.68 ± 7.87	885. 36 ± 18
Gauze + chitosan	3.38 ± 0.11	744.94 ± 12
Gauze (He pretreated) + chitosan	4.88 ± 0.07	754.46 ± 17
Gauze (He/O_2 pretreated) + chitosan	8.76 ± 0.20	766.36 ± 19

moisture results in wound desiccation; both conditions have been demonstrated to delay wound healing.[35] Compared to a wound left open to heal in air, a wound occluded under a transparent film has a higher rate of epithelialization.[36] Gupta et al.[37] studied the air permeability of cotton fabric and cotton fabric covered with chitosan film. It was observed that air permeability of ~8.6 $cm^3/cm^2/sec$ would help in maintaining the wound moist without inducing maceration.

Gauze with no chitosan nanofibers showed an average air permeability of 265.68 $cm^3/cm^2/sec$. Untreated, He- and He/O_2-plasma treated substrates with chitosan nanofibers showed reduced average air permeabilities of 3.38, 4.88, and 8.76 $cm^3/cm^2/sec$, respectively (Table III). As all the values of air permeabilities obtained after deposition of nanofibers onto the substrate have values close to the 8.6 $cm^3/cm^2/sec$ recommended by Gupta et al.,[37] the composite bandages would help in maintaining the appropriate wound moisture. Thus, the use of chitosan nanofiber coated gauze bandages should result in faster wound healing. It is expected that the air permeability would be affected by the thickness of nanofibers deposited onto the gauze fabric. Higher thicknesses of nanofibers will result in lower air permeabilities due to the greater number of nanofiber layers making the composite bandage a more compact structure.

Moisture Vapor Transmission Rate

Bolton[38] studied a variety of dressings and determined that an MVTR of less than 840 $g/m^2/day$ is required to maintain a moist wound surface. MVTRs for gauze with no nanofibers, and untreated gauze and plasma pretreated gauze with chitosan nanofibers are shown in Table III. A higher MVTR (885.36 $g/m^2/day$) was obtained in case of substrate with no chitosan nanofibers than for the untreated as well as the He, He/O_2 plasma treated gauzes with chitosan nanofibers deposited on them (744.94, 754.46, 766.36 $g/m^2/day$, respectively). The decrease in the MVTR after deposition of the chitosan nanofiber layer as compared with the gauze alone indicates that the composite bandage is likely to provide better wound moisture retention than the untreated gauze.

XPS Analysis of Substrate after Plasma Treatment

The carbon content in the control sample was 84.88% as shown in Table IV. Typical cellulose would have a carbon content of 66.9%.[26] This uncommon cellulose composition may be due to the presence of residual long chains of hydrocarbons[39] on the cotton fiber surface which remain even after aqueous processing, or may be due to the composition of the cotton fiber outer wall which consists of mixture of fats, waxes, and resin.[40] XPS analysis of control gauze, He-, and He/O_2-plasma treated gauze

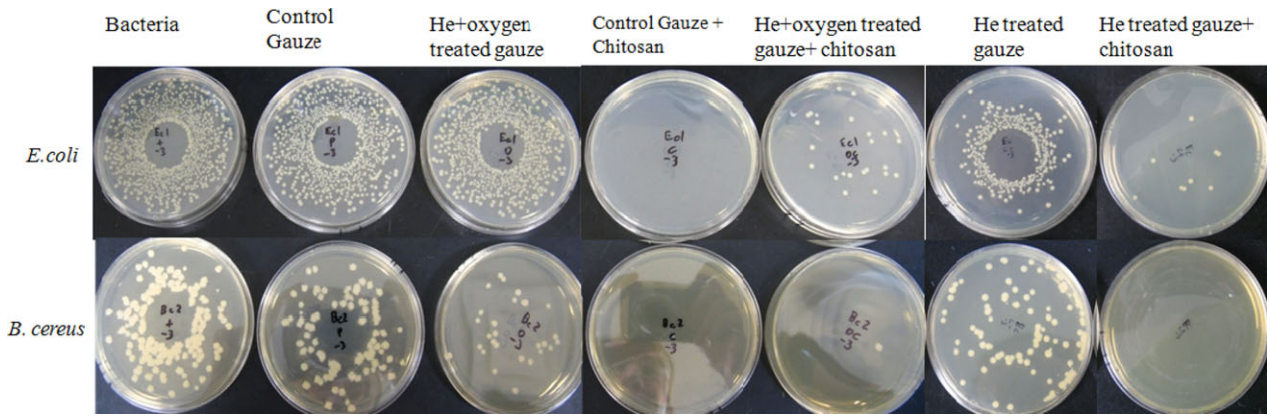

Figure 5. Antibacterial testing on control (untreated) gauze, He, and He/O_2 treated substrate with and without chitosan nanofibers. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

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Table IV. Results of Surface Elemental Analysis for Plasma Treated and Untreated Cotton Substrate with Atmospheric Pressure Plasmas

100% cotton substrate	C-spectra			Overall	
	C—C (%)	C—O (%)	COO—(%)	O (%)	C(%)
No treatment	85	14	1	15.12	84.88
He Plasma treated	82	9	9	22.38	77.62
He-O_2 plasma treated	82	8	10	18.82	81.18

was carried out to study the plasma effect on surface chemistry of the treated substrates. Previous studies in our lab showed possible radical formation on the surface of cotton fabric due to fluorinated radio frequency plasma.[26] Table IV shows a reduction in the carbon content of the cotton substrate from 84.88% to 77.62% and 81.18% after He- and He/O_2-plasma treatment. This effect was more pronounced for the He treated substrate than for the He/O_2-plasma treated substrate. Reduction in surface carbon content is due to the etching of some of the long chain hydrocarbons from the surface as a result of plasma treatment which results in roughness of the surface. Oxygen contents of the cotton substrate were increased from 15.12% to 22.38% and 18.82% after He- and He/O_2-plasma treatments, respectively. Higher oxygen content can be explained by the scission at site "B" from the cellulosic ring as shown in Figure 6. Scission at sites "C" and "E" would produce radicals that could react with active groups of the chitosan nanofibers. In addition, dehydrogenation or dehydroxylation at site "A" and "B" in —C—O—H groups (Figure 6) on cellulose can make the cotton substrate susceptible to reaction with chitosan nanofibers at C6. Due to the abundance of hydroxyl groups on chitosan as well as on cotton substrate, there are high chances of hydrogen bonding at the nanofiber–substrate interface.

C1s spectral analysis showed that there was formation of new functional groups on the cotton substrates after treating them with He- or He/O_2-plasma. Increases in COO— (289.1 eV)

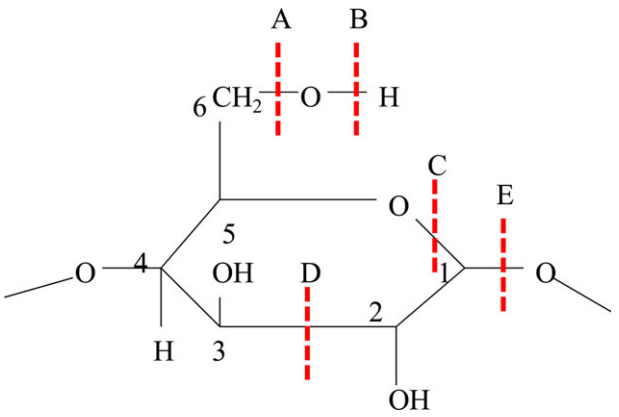

Figure 6. Possible sites for scission of cotton substrate after plasma treatment: (A) dehydroxylation, (B) dehydrogenation, (C) scission between C1 and ring oxygen, (D) dehydrogenation/dehydroxylation, (E) scission between C1 and glycosidic oxygen. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Table V. Absorbency of Gauze and Composite Wound Dressing

Samples	Average absorbency (g)	Absorbency (%)
Gauze	0.12 ± 0.01	91 ± 6
Gauze + chitosan	0.22 ± 0.01	153 ± 10
Gauze (He pretreated) + chitosan	0.22 ± 0.01	164 ± 7
Gauze (He/O_2 pretreated) + chitosan	0.23 ± 0.01	166 ± 9

indicate formation of polar groups available for crosslinking with hydroxyl or cationic amine groups of chitosan nanofibers. After treating the cotton substrate with plasma, breaking of cellulose ring can occur resulting from scission at sites "C" and "E". This scission can be explained by the reduced intensities of C—C and C—O bonds in C1 spectra and can be responsible for crosslinking between the substrate and nanofibers to be deposited on it. Considering all these factors, it is likely that increased functionality and surface roughness as a result of plasma treatment are responsible for the increased adhesion. Qualitative assessment of the surface roughness of nanofibers is technically challenging and was not characterized in this study.

Absorbency

Adding a nanofiber layer onto the conventional cotton gauze is expected to result in an increase in the liquid absorbency. Due to the high surface area to volume ratio of the nanofibers, they exhibit water absorptions in the range of 17.9–213%.[41] Absorbency of gauze and composite bandages is shown in Table V. The chitosan nanofibers composite wound dressing showed an average of 68–82% higher absorbency than the gauze fabric alone. Though it was expected that plasma treatment would make the cotton substrate more hydrophilic, there was no significant dependence of absorbency on the type of plasma used to treat the composite bandages.

These composite wound dressings of cotton gauze and chitosan nanofibers could potentially help resolve excessive hydration and wound maceration by reducing swelling and inflammation through higher absorbency. Chitosan nanofiber coated composite wound dressings showed absorbencies of 0.22–0.23 g as shown in Table V. These results are comparable to those of commercial dressings, e.g., Arglaes and Acticoat7,[42] which have absorbencies of 0.5 and 0.6 g, respectively. It is expected that the absorbency can also be manipulated by the thickness of nanofiber layer. If a thicker layer of nanofiber is deposited on the substrate, the absorbency of composite wound dressings can be increased.

CONCLUSIONS

Chitosan nanofibers were electrospun onto 100% cotton gauze substrates to form composite bandages. Atmospheric pressure plasma technology was utilized to improve the adhesion and durability of the nanofiber coating. Peel tests showed that treatment of the substrate with 100% He plasma and 99% He/1% O_2 plasmas increased the adhesion between nanofiber layers and substrates by up to four times. Also, plasma pretreatment of the

gauze fabric prior to electrospinning significantly reduced degradation of the nanofiber layer due to repetitive flexing. Antibacterial results showed that with plasma pretreatment of the substrate, the antibacterial properties of chitosan were retained against Gram positive (*B. cereus*) and Gram negative bacteria (*E. coli*). Air permeability and moisture vapor transport were reduced due to the presence of a nanofiber layer upon the substrate, which is expected to aid in keeping the wound moist, resulting in better wound healing. XPS analysis showed that after the substrate was treated by plasma, the surface carbon content (C1s) was decreased and oxygen content was increased. Also, plasma treatment created surface oxidation, which was responsible for the increased adhesion between the substrate and chitosan nanofibers through crosslinking between the active sites. As compared to uncoated gauze alone, gauze coated with chitosan nanofibers had on average a 68–82% higher absorbency, which is likely to enhance absorption of wound exudates and blood.

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