Improved Antimicrobial Activity of Polypropylene and Cotton Nonwoven Fabrics by Surface Treatment and Modification with Chitosan

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ABSTRACT: Nonwoven polypropylene and cotton fabrics were subjected to plasma pretreatment followed by flash evaporation and radiation crosslinking acrylate polymer coating, which is based on a vacuum deposition, solvent free, process that produces high quality, uniform fabrics with various thicknesses (0.05–5.0 μm). These treated fabrics were then dipped into chitosan, carboxymethyl chitosan, and carboxymethyl chitin solution. These polysaccharides form strong complexes with the modified surface. The antimicrobial activity of these treated samples was then evaluated for their antifungal and antibacterial properties. The antifungal activity for *Fusarium oxysporum* f. sp. *lycopersici, Verticillium albo-atrum*, and *Alternaria solani* (*A. alternata*) were examined by the disc plate method. The antibacterial activities of the modified fabrics against *Clavibacter michiganensis and Pseudomonas solanacearum* were also examined by the viable cell counting method. The inhibition zone of the chitosan covered samples has increased by a factor of 2–3.1 over the original pretreated samples. The chitosan-modified fabrics showed a good antibacterial activity in killing almost 10^5 cells/mL within 18–23 h. © 2008 Wiley Periodicals, Inc. J Appl Polym Sci 108: 2290–2296, 2008

Key words: nonwoven polypropylene and cotton fabrics; surface modification; chitosan; antimicrobial activity

INTRODUCTION

Food quality and safety are major concerns in the food industry as consumers prefer fresher and minimally processed products. In particular, bacterial and fungal contamination of ready-to-eat products is of concern to human health.

The major portion of food spoilage originates from microbial contamination on the food surface; the antimicrobial packaging will provide an appropriate way of solving the food contamination surface.

Antibacterial sprays or dips have been done to overcome those contaminations.[1] However, direct surface application of antibacterial substances has some limitations because the active substances could be neutralized, evaporated or diffused inadequately into the bulk of food.[2,3] Edible fabrics or coatings have been investigated for their abilities to retard moisture, oxygen, aromas, and solute transports.[4,5] This is further improved by film carrying food additives, such as antioxidants, antimicrobial, colorants, flavors, fortified nutrient, and spices.[6,7] In many cases, the agents being carried are slowly released into the food surface and therefore remain at high concentration for extended period of time.[1,8]

Polyethylene (PE) and polypropylene (PP) are engineering plastics widely used in many engineering and biomedical applications. By appropriate surface treatments PE and PP can be rendered biocompatible and acquire antimicrobial properties. Different methods for polymer surface modifications have been attempted such as plasma-treatment techniques.[9] The flash evaporation vacuum polymer coating process has shown great success in functionalizing surfaces of fabrics, woven and nonwoven fabrics, paper, and foam substrates. Properties such as oil and water repellency and wettability, antibacterial and other chemical functionalities are easily achievable with thin coatings.[10–13]

Blending with other macromolecules[14,15] and immobilization of small or large molecules on the surface have been attempted to obtain materials with much improved biocompatibility and better hydrophilic surfaces. The change in surface properties was found to affect the interaction of the surface with the surroundings.[16,17]

The present study was conducted to modify the surface of nonwoven PP and cotton fabrics to impart antimicrobial potential onto them.

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The nonwoven fabrics were subjected to plasma irradiation, and then were treated with chitosan, CM chitosan, and CM chitin solutions. One of the reasons for the antimicrobial character of chitosan is its positively charged amino group, which interacts with the negatively charged microbial cell membranes, leading to the leakage of proteinaceous and other intracellular constituents of the microorganisms.[18,19] A number of studies on the antimicrobial characteristics of films made from chitosan have been carried out earlier.[1,20,21]

The antifungal activities of the modified membranes against *Fusarium oxysporum* f. sp. *lycopersici*, *Verticillium albo-atrum*, and *Alternaria solani* (*A. alternata*) were examined by the disc plate method. The antibacterial activities of the modified membranes against *Clavibacter michiganensis and Pseudomonas solanacearum* were also examined by the viable cell counting method.

MATERIALS AND METHODS

Materials

Nonwoven fabrics

PP nonwoven fabrics and cotton nonwoven fabrics were obtained as a kind gift from Sigma (referred to as SMS for the PP samples).

Plasma treatment

(According to Sigma Technologies, Intl., Tucson, AZ)

This is an in-line process with very short time (ms) between plasma treatment and deposition of a liquid monomer layer.[10–13]

The fabrics were pretreated with plasma Ar/O_2, 1 kW. The role of plasma here is to clean the surface and to eliminate any impurities. Then the treated fabrics are coated with ζ-60-20-20 carboxylic functional crosslinked thermoset acrylate by a flash evaporation, vacuum deposition followed by radiation curing with an electron beam, 10 kV, 100 mA.

The deposited layer of crosslinked polymer is attached to the fabric with covalent bonds that lead to strong durable coatings, which can resist degradation from washing and cleaning cycles. This technique combines the advantages of plasma treating (covalent bonding to the substrate) and thin polymer film coating on highly activated surfaces.

Condensing the monomer/oligomer vapor on the plasma treated fibers forms a homogeneous thin liquid layer that covers the entire surface of each individual fiber without connecting fiber to fiber and blocking the pores (0.3–0.5 μm thin film). This maintains the porosity and breathability of the substrate.

Chitosan and derivatives

Chitosan was extracted from red shrimp. The exoskeleton of the shrimp was crushed and treated with HCl, NaOH 1–2*M*, to extract the chitin then with 40% NaOH to deacetylate it into chitosan. The extracted chitosan was characterized as for the degree of deacetylation (using elemental analysis and potentiometric titration) and molecular weight by measuring the intrinsic viscosity.[22] Carboxymethyl chitosan (CM chitosan) and chitin (CM chitin) were prepared according to the method in Ref. 23 both were water soluble. The degree of substitution was determined by a potentiometric titration according to Ref. 24 and was found to be 0.70.

Method

PP fabrics were immersed into the acidic chitosan solution for different periods of times, 3–24 h. After removing from the chitosan solutions the fabrics were either dried with slight pressing with a filter paper or washed several times with distilled water then dried or dipped into aqueous glutaraldehyde solution at pH 7 to induce crosslinking of the chitosan layer to prevent its leakage easily from the surface.

Fourier transforms infra-red FTIT

The Fourier transforms infra red FTIR spectra were measured for the fabrics in the transmission mode in the range 400–4000 cm^{-1}, using Perkin–Elmer 2000 spectrophotometer. The attenuated total reflectance ATR method was not successful since the fabrics have tiny holes, which interrupted and prevented the reflectance of the IR beam.

Pathogenic microorganisms and culture conditions

All strains of fungi and bacteria used in this study were maintained as pure cultures. The fungi *Fusarium oxysporum* SCHL., f. sp. *lycopersici,* (SACC.) Snyder and Hansen (ATCC 64,987), causing *Fusarium* wilt and *Verticillium albo-atrum* Reinke and Berthold (NRRL 1204), causing *Verticillium* wilt, were maintained on sarcina agar,[1,25] *Alternaria solani (A. alternate*) (ELL. and MART.) Jones and Grout (NRRL 2168), causing early blight, was maintained on Czapek–Dox agar.

The pathogenic bacteria *Clavibacter michiganensis* ssp. *Michiganensis* (E.F. Smith) (NRRL B-33), causing bacterial canker, and *Pseudomonas solanacearum* (E.F. Smith) (NRRL B-3312), causing bacterial wilt, was maintained on nutrient agar.

Bioassay for antifungal activity

The susceptibilities of the test fungal spores [*Fusarium oxysporum, Verticillium albo-atrum*, and *Alternaria solani (A. alternata)*] as seeded in Dox's medium on sterilized discs (6 mm) of different kinds of the PP polymers, were determined according to the method proposed by Olurinola et al.[26] The sterilized membrane discs were placed on the surface of the seeded Dox medium in triplicates. Plates were allowed to stand for 2 h to allow for the diffusion. Then the plates were incubated at 28°C for 48 h, after which the susceptibility of each organism to each membrane sheet was estimated by measuring the diameter of the inhibition zones.

Antibacterial assessment

The bactericidal activity was evaluated based on the killing rate by the viable cell counting technique[27] against *Clavibacter michiganensis and Pseudomonas solanacearum.* One loopful of the bacteria was inoculated into 10 mL of nutrient agar and incubated at 37°C for 18–24 h, and then 20 mL of phosphate buffer solution (PBS) (composed of 0.2*M* Na_2HPO_4, 0.2*M*NaH_2PO_4, 0.5 g NaCl, and 2 g/L Tween-80 to 1000 mL) was added. After mixing, 1 mL of the solution was added to 9 mL of the nutrient broth and mixed with vortex mixer. The bacteria solution was further diluted with PBS to 1.5×10^5 cells/mL, and placed in flasks (0.4 g/sample of each group). After incubating for 0–24 h at 37°C, 20 mL of PBS were added and stirred for 30 s. Consecutive dilutions were repeated by taking 1 mL of the previous solution and mixing with 9 mL of PBS. From the diluted solution the surviving bacteria were determined by the spread plate method. After inoculation, the plates were kept at 28°C and the colonies were counted after 18–24 h.

Statistics

All measurements are the mean of five replicates; the results obtained were processed by analysis of variance and the significance was determined at the least significant difference levels of 1 and 5%. That is to say that the probability $P < 0.05$ means a significant figure and $P < 0.01$ means highly significant value as compared with the control material.

RESULTS AND DISCUSSION

FTIR and surface characterization of the PP nonwoven fabric

Figure 1(a) illustrates the spectra of the original PP fabric with the characteristic bands at 1461.6 and 1378.4 cm^{-1} due to the asymmetric and symmetric bending vibration of the CH_3 and CH_2, respectively. The bands at around 3000 cm^{-1} are due to the stretching vibration of the tertiary hydrogen. Figure 1(b) shows the spectrum after plasma treatment. It shows bands at 1763 and 1705 cm^{-1} indicating the formation of carbonyl or carboxyl groups, the bands at 3000 cm^{-1} showed splitting and shifting indicating as expected changes in the tertiary H atom. While Figure 1(c) shows a small shoulder band at 3400–3380 cm^{-1} due to —OH and —NH_2 stretching of chitosan, these fabrics are quite thick therefore the chitosan concentration is relatively very small compared with the PP bulk. The band at 3380 cm^{-1} indicates that the OH and NH_2 stretching bands are sifted to lower wave number presumably because of the association with the reactive sites on the PP fabrics. Bands at 1646 and 1560 cm^{-1} are characteristic to the amide band also indicative of chitosan adsorption. Scheme 1 is a possible presentation to the inter-

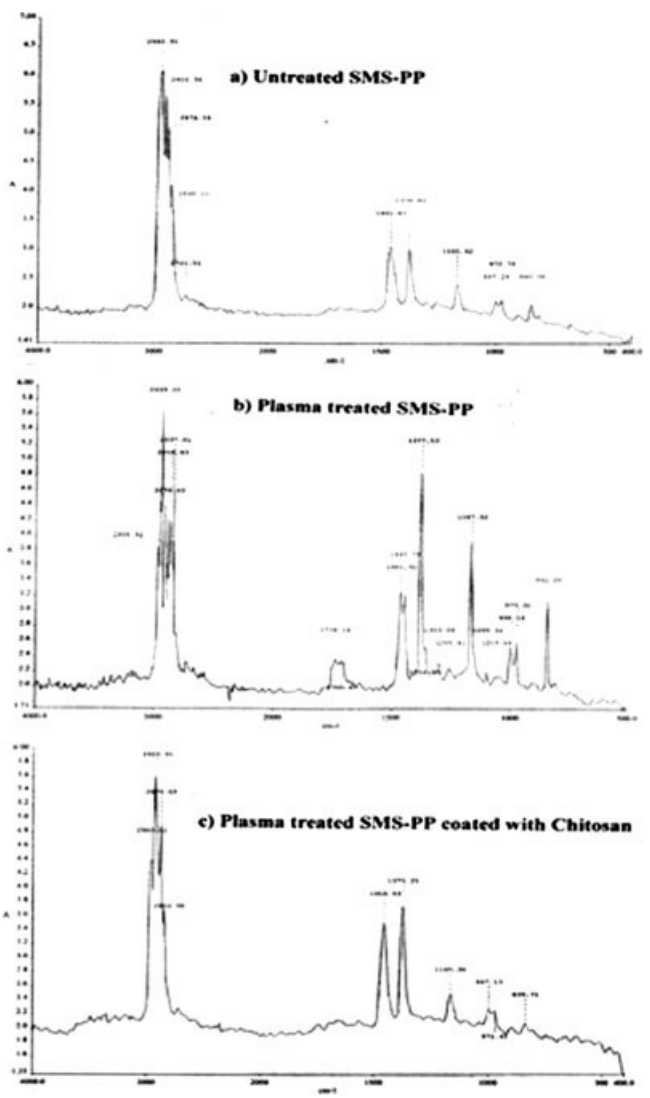
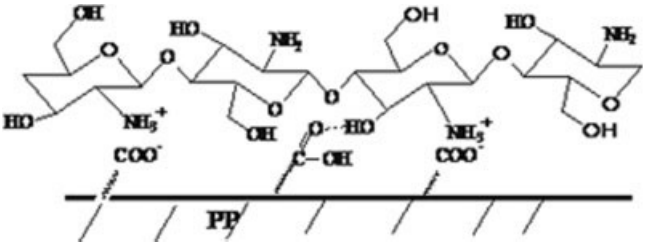


Figure 1 FTIR curves for (a) PP control, (b) plasma treated, and (c) plasma/chitosan treated.



Scheme 1 Representation showing the association of pretreated fabric surface with chitosan.

action of chitosan with the plasma treated and acrylate covered PP fabrics. The carboxyl groups onto the surface will associate with the NH_2 groups and the OH groups in the chitosan moiety. Further evidence of the modification of the PP surface was tested by drops of water as seen in Figure 2. Figure 2(a) shows that the water drop onto the original untreated PP film is quite spherical indicating very small wetting with the PP surface and reflecting the hydrophobic nature of its surface even if left for a long time (several hours). Figure 2(b) shows the drop onto the plasma treated PP film, the drop shows a distorted sphere because of the increased wetting of the surface, and according to the manufacturer that plasma treatment increases hydrophilicity of the surface. However a complete and instantaneous wetting was observed after immersing the plasma treated fabric into chitosan solution as shown in Figure 2(c).

The cotton nonwoven fabrics have quite hydrophilic surfaces even the original untreated ones.

Antifungal activity

Table I contains the characteristics of the investigated samples.

Table II shows the antifungal activity of PP fabrics treated with plasma then with chitosan, CM chitosan, and CM chitin (samples A, B, C, D, E, F, G, H, and I) against some fungal pathogens. The effectiveness was found to depend on the type of fabrics, treatment, and the tested fungus. The effect was observed by the diameter of the inhibition zones. The results obtained revealed that the untreated PP fabrics had no inhibitory effect at all against all the tested fungal species. The plasma treated fabrics showed slight or negligible effectiveness on the other hand, fabrics treated with plasma then with chitosan showed pronounced antifungal efficacy against all the tested fungal species. The fungitoxic effect of the plasma/chitosan treated fabrics for *Verticillium albo-atrum* and *Alternaria solani* (*A. alternata*) was 2.6 times higher than the value for the plasma exposed fabrics. Meanwhile, the *Fusarium oxysporum* possessed lower antagonistic activity (plasma/chitosan treated fabrics are 2.0 times higher than that of the plasma exposed fabrics). The PP fabrics treated with plasma then dipped in chitosan solution were pressed gently by filter paper then tested, another sample was washed with distilled water several times before measurement and a third sample was dipped into 1% glutaraldehyde GA solution at pH 7. Washing with sterilized distilled water (Sample D) did not lead to dramatic reduction of the antifungal activity, which means that the antifungal efficiency is not due to the presence of acetic acid but due to the chitosan itself, which must be adsorbed by strong forces of interaction (Scheme 1). A slight enhancement of the activity was observed when the fabrics were treated with glutaraldehyde, which could be a consequence of the increasing of the hydrophobic nature of the crosslinked chitosan layer.[28] The same trend of fungicidal behavior was observed in the case of CM chitosan. Further enhancement of the activity up to 3.1 times higher than the plasma treated fabrics was obtained after GA treatment of the CM chitosan sample. The highest activity was observed when CM chitin treated sample was dipped in GA leading to an enhancement factor 3.2 for *V. albo-atrum*. The data of the antifungal activity of the cotton samples treated with plasma then in chitosan solution followed by glutaraldehyde solution are depicted in Table III. Samples treated with chitosan, CM chitosan, and CM chitin showed a similar antifungal efficiency as those of the PP treated fabrics. Whereas when the cotton samples were treated with solution of chitosan-*g*-polyvinyl pyridine copolymer a much higher efficiency was observed, this is in accord with a previous work regarding the high antifungal activity of this graft copolymer.[29,30]

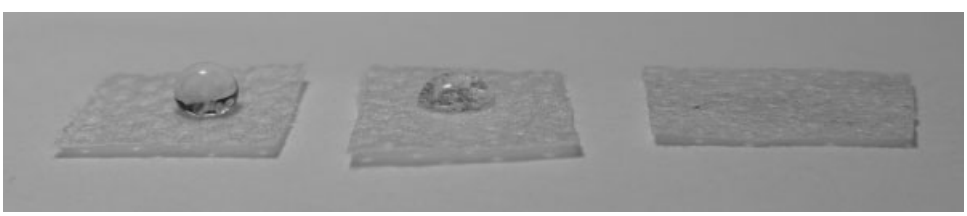


Figure 2 (a) Drop of distilled water on untreated PP (b) water on plasma treated PP, (c) PP treated/chitosan film the water is spread completely.

TABLE I
Characteristics of the Investigated Samples

Sample	Characteristics
A	Untreated polypropylene nonwoven fabric
B	Plasma treated nonwoven fabrics, SMS
C	Sample B dipped in chitosan for 3 h
D	Sample C washed with distilled water
E	Sample C treated with GA (1%) solution pH 7
F	CM RS chitosan/SMS dried only
G	Sample G dipped in GA
H	CM chitin/SMS dried only
I	Sample H dipped in GA solution
J	Cotton fabric immersed in chitosan RS/GA
K	Cotton fabric in CM chitosan/GA
L	Cotton fabric CM chitin/GA
M	Cotton fabric/95% graft VP/chitosan

CM RS, carboxymethyl chitosan (chitosan extracted from red shrimp); CM/H_2O, carboxymethyl chitosan washed with water; CM/GA, carboxymethyl chitosan treated with glutaraldehyde.

The antagonistic activities of many biomembranes against different pathogenic microorganisms *in vitro* are widely recognized.[31,32]

Antibacterial activity

Figures 3 and 4 show plots of the logarithm of the number of viable cells [log (CFU/mL)] as function of time of *Clavibacter michiganensis and Pseudomonas solanacearum* after contact with native, plasma exposed, and chitosan/plasma treated PP nonwoven fabrics (CFU stands for colony forming units). Only the last membrane exhibited a biocidal ability. The native polymer did not show any bactericidal property at all on the contrary the viable cell number of the bacteria increased. The second film (plasma exposed) showed lower viable cell number with no noticeable bactericidal property.

On the other hand, the chitosan/plasma treated film showed a pronounced decline of the cells with complete annihilation of the bacterial cells (about 10^5 cells/mL) after 16 and 19 h for *Clavibacter michiganensis and Pseudomonas solanacearum*, respectively. It seems that *Pseudomonas solanacearum* is slightly more tolerant to the effect of chitosan than the other species.

The mode of action of the biocides can be interpreted as follows[33]: The bacterial cell surfaces are known to be negatively charged while the chitosan is positively charged because of the presence of the protonated amino groups, therefore the bacteria cell wall will be adsorbed on the cationic surface of the chitosan membrane, which binds and disrupt the cytoplasm membrane of the bacteria.[34] This causes the release of cytoplasmic constituents, such as the

TABLE II
Antifungal Efficacy of the Polypropylene (Native, Plasma Exposed, and Chitosan/Plasma treated) Nonwoven Films on the Growth of *Fusarium oxysporum, Verticillium albo-atrum*, and *Alternaria solani* (*A. alternata*) after 48 h of Incubation at 28°C by a Disc Plate Method

Samples	*Fusarium oxysporum*	*Verticillium albo-atrum*	*Alternaria solani*
A	0	0	0
B	11.3	12.7	15.2
C	22.5	33.3	39.7
D	21.6	32.4	37.8
E	24.4	37.6	45.5
F	22.8	33.5	40.2
G	24.9	39.8	46.1
H	23.1	33.9	40.8
I	25.1	40.2	46.8
LSD (%)			
5	1.9	1.8	2.1
1	3.4	3.1	3.6

Mean values of the diameter of the inhibition zones in mm.

TABLE III
Antifungal Efficacy of the Cotton Fabrics (Native, Plasma Exposed, and Chitosan/Plasma Treated) on the Growth of *Fusarium oxysporum, Verticillium albo-atrum*, and *Alternaria solani* (*A. alternata*) after 48 h of Incubation at 28°C by a Disc Plate Method

Samples	*Fusarium oxysporum*	*Verticillium albo-atrum*	*Alternaria solani*
J	22.3	32.9	39.6
K	23.6	35.8	43.1
L	24.9	36.3	44.8
M	26.4	40.9	50.3
LSD (%)			
5	1.9	1.8	2.1
1	3.4	3.1	3.6

Mean values of the diameter of the inhibition zones in mm.

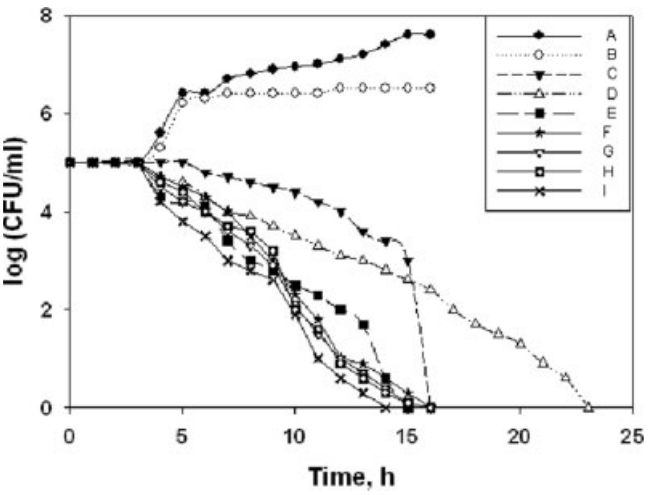


Figure 3 Viable cell number of *Clavibacter michiganensis* as a function of time grown on nonwoven PP fibers. Description of the letters is found in Table I.

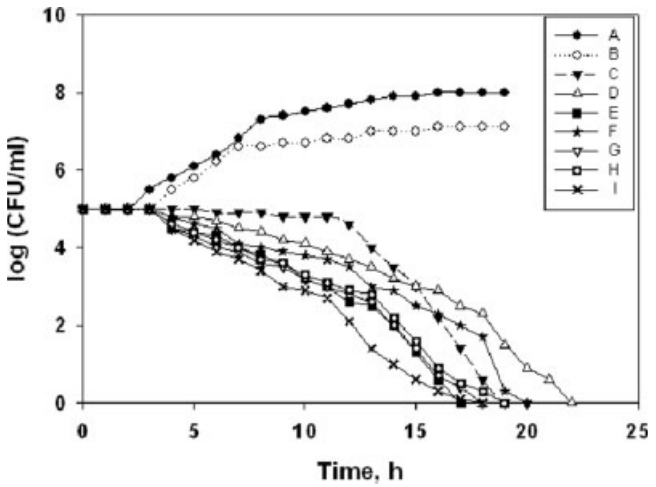


Figure 4 Viable cell number of *Pseudomonas solanacearum* as a function of time grown on nonwoven PP fibers, the same symbols as in Figure 2.

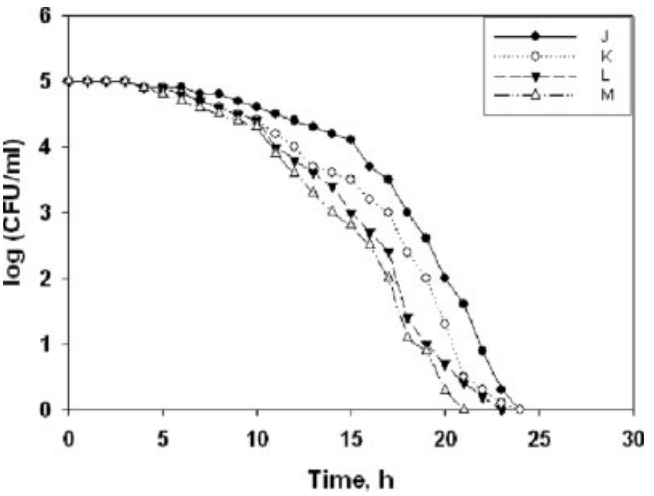


Figure 6 Viable cell number of *Pseudomonas solanacearum* as a function of time grown on cotton fibers.

DNA and the RNA, to take place continuously and eventually leading to the death of the bacteria.

Thus the chitosan/plasma membrane can be considered as an antimicrobial material. Liu et al.[35] also indicated that the antimicrobial activity of chitosan oligomers seems to be caused mainly by the inhibition of the transcription from DNA. It has been also reported that the *N*-acetylglucosamine unit of the chitosan molecule is even more bioactive than the glucosamine residue in certain cases.[36] The CM chitosan and CM chitin have been found to exhibit comparable antibacterial activity as chitosan itself.

Figures 5 and 6 illustrate the viable cell number of *Clavibacter michiganensis and Pseudomonas solanacearum* after contact with cotton nonwoven fabrics. The plasma treated fabrics were then coated with chitosan then dipped in glutaraldehyde at pH 7 also in CM chitosan and CM chitin, another sample was immersed in acetic acid (1%) solution of (chitosan-*g*-polyvinyl pyridine) copolymer. All the coated fabric fabrics showed antibacterial activity with complete elimination of the bacteria after ~18–23 h, which indicates fairly good antibacterial characteristics. The figures indicate that the chitosan coated cotton nonwoven fabrics showed lower potency as antibacterial material than the corresponding PP fabrics. Therefore the PP fabrics are more suitable as active packaging system than the cotton one.

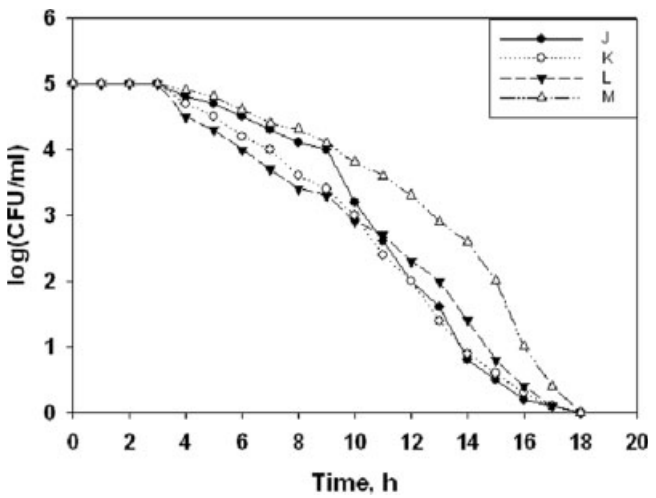


Figure 5 Viable cell number of *Clavibacter michiganensis* as a function of time grown on cotton fibers.

CONCLUSIONS

Two types of materials, nonwoven PP and cotton fabrics were treated with plasma and subjected to flash evaporation and radiation crosslinking for polymer coating based on a vacuum deposition, solvent free, process that produces high quality, uniform fabrics with various thicknesses and were then dipped in chitosan, CM chitosan, and CM chitin solutions. A chitosan-*g*-polyvinyl pyridine copolymer solution was also tested in the same way. The chitosan was found to adhere to the fabrics imparting a biocidal activity onto them. The bioassay of the fabrics was carried out using several microorganisms. It was found that the native fabrics had no biocidal activity, the plasma treated ones showed slight activity however the fabrics coated with chitosan and its derivatives showed much higher antifungal as well as anti bacterial activity. The antimicrobial activity was found to depend on the type of organism and the type of chitosan derivative. Therefore, treatment with chitosan and its derivatives can be considered as a practical method to impart antimicrobial properties onto PP and cotton fabrics, which have impor-

tant practical usage. These fabrics can be used for food and seeds packaging applications. This will be considered in a future communication.

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References

1. Ouattara, B.; Simard, R. E.; Piette, G.; Begin, A.; Holley, R. A. J Food Sci 2000, 65, 768.
2. Park, S. Y.; Marsh, K. S.; Rhim, J. W. J Food Sci 2002, 67, 194.
3. Torres, J. A.; Motoki, M.; Karel, M. J Food Process Preserv 1985, 9, 75.
4. Gennadios, A.; Weller, C. L. Food Technol 1990, 44, 63.
5. Pena, D. C. R.; Torres, J. A. J Food Sci 1991, 56, 497.
6. Han, J. H. Food Technol 2000, 54, 56.
7. Coma, V.; Sebti, I.; Pardon, P.; Deschamps, A.; Pichavant, F. H. J Food Prot 2001, 64, 470.
8. Durango, A. M.; Soares, N. F. F.; Andrade, N. J. Food Control 2006, 17, 336.
9. Ferrero, F.; Bongiovanni, R. Surf Coat Technol 2006, 200, 4770.
10. Mikhael, M. G.; Ellwanger, R. E.; Yializis, A. In Proceedings of Thirteenth International Conference on Vacuum Web Coating; Bakish, R. Ed.; Bakish: Tucson, 1999.
11. Yializis, A.; Mikhael, M. G.; Ellwanger, R. E.; Mount, E. M., III. In 42nd Annual Technical Conference Proceedings; Society of Vacuum Coaters: Chicago, 1999; p 469.
12. Yializis, A.; Mikhael, M. G.; Ellwanger, R. E. In Proceedings of Twelfth International Conference on Vacuum Web Coating; Bakish, R., Ed.; Bakish: Reno, 1998; p 179.
13. Yializis, A.; Mikhael, M. G.; Miller, T. A.; Ellwanger, R. E. In Proceedings of Eleventh International Conference on Vacuum Web Coating; Bakish, R.; Bakish: Miami 1997; p 138.
14. Kiss, E. Bertoti, I.; Vargah-Butler, E. I. J Colloid Interface Sci 2001, 245, 91.
15. Mao, C.; Yuan, J.; Mei, H.; Zhu, A.; Shen, J.; Lin, S. Mater Sci Eng C 2004, 24, 479.
16. Qu, X.; Wirse, A.; Olander, B.; Albertsson, A.-C. Polym Bull 2001, 46, 223.
17. Higuchi, A.; Shirano, K.; Harashima, M.; Yoon, B. O.; Hara, M.; Hattori, M.; Imamura, K. Biomaterials 2002, 23, 2659.
18. Shahidi, F.; Arachchi, J. K. V.; Jeon, Y. J. Trends Food Sci Technol 1999, 10, 37.
19. Blac k, J. G. Microbiology: Principles and application; Prentice-Hall: New Jersey, 1996; p 80.
20. Chen, M. C.; Yeh, G. H. C.; Chiang, B. H. J Food Process Preserv 1996, 20, 279.
21. Coma, V.; Martial-Gros, A.; Garreau, S.; Copinet, A.; Deschamps, A. J Food Sci 2002, 67, 1162.
22. Domard, A.; Rinaudo, M. Int J Biol Macromol 1983, 5, 49.
23. Chen, X. G.; Park, H. J. Carbohydr Polym 2003, 53, 355.
24. Ge, H. C.; Luo, D. K. Carbohydr Res 2005, 340, 1351.
25. Gibriel, A. Y.; Magdoub, M. N. I.; El-Nawawy, M. A.; Rizk, I. R. S.; Fayed, E. O. Catalogue of Strains (CAIM), 2nd ed.; Microbiological Ressource Center (MIRCEN), Faculty of Agriculture, Ain-Shams University, Cairo, Egypt, 1987.
26. Olurinola, P. F.; Ehinmidu, J. O.; Bonire, J. J. Appl Environ Microbiol 1992, 58, 758.
27. Park, J. S.; Kim, J. H.; Nho, Y. C.; Kwon, O. H. J Appl Polym Sci 1998, 69, 2213.
28. Tangpauthadol, V.; Pongchaisiikul, N.; Hoven, V. P. Carbohydr Res 2003, 338, 937.
29. Elkholy, S.; Khalil, K. D.; Elsabee, M. Z. J Appl Polym Sci 2006, 99, 3308.
30. Elkholy, S.; Khalil, K. D.; Elsabee, M. Z.; Eweis, M. J Appl Polym Sci 2007, 103, 1651.
31. Azad, A. K.; Sermsintham, N.; Chandrkrachang, S.; Stevens, W. F. J Biomed Mater Res B Appl Biomater 2004, 69, 216.
32. Yang, F.; Li, X.; Cheng, M.; Gong, Y.; Zhao, N.; Zhang, X. J Biomater Appl 2002, 16, 215.
33. Kato, K.; Ikada, Y. Biotechnol Bioeng 1998, 51, 581.
34. Gatenholm, P.; Ashida, T.; Hoffman, A. S. J Polym Sci Part A: Polym Chem 1997, 35, 1461.
35. Liu, X. F.; Guan, Y. L.; Yang, D. Z.; Li, Z.; You, K. D. J Appl Polym Sci 2001, 79, 1324.
36. Peluso, G.; Petillo, O.; Ranieri, M.; Santin, M.; Ambrosic, L.; Calabró, D.; Avallone, B.; Balsamo, G. Biomaterials 1994, 15, 1215.
37. Anderson, D.; Nguyen, T.; Lai P.-K.; Amiji, M. J Appl Polym Sci 2001, 80, 1274.