Surface Grafting of Polyester Fiber with Chitosan and the Antibacterial Activity of Pathogenic Bacteria

S.-G. Hu, C.-H. Jou, M.-C. Yang

Department of Fiber and Engineering, National Taiwan University of Science and Technology, Taipei, Taiwan 10672

Received 12 November 2001; accepted 1 April 2002

ABSTRACT: Three polyesters—poly(ethylene terephthalate), poly(2-methyl-1,3-propylene terephthalate-*co*-ethylene terephthalate), and poly(1,4-cyclohexylene terephthalate-*co*-ethylene terephthalate)—were preirradiated with ^{60}Co-γ-rays. Then, acrylic acid and *N*-vinylformamide were grafted to these irradiated fibers. Fibers grafted with *N*-vinylformamide were further hydrolyzed with acid so that the amide groups would convert into amino groups, and they were treated with glutaraldehyde so that aldehyde groups would be introduced. Chitosan or chitooligosaccharide was then grafted to these fibers via either esterification or imine formation. Four pathogenic bacteria—methicillin-resistant *Staphylococcus aureus*-1 (MRSA), *Staphylococcus aureus*-2, *Escherichid coli*, and *Pseudomonas aeruginosa*—were tested to determine the antibacterial activities of chitosan-grafted and chitooligosaccharide-grafted fibers. The results showed that grafting chitosan via imine formation could achieve a higher surface density for amino groups and give higher antibacterial activity to those four bacteria tested. The antibacterial activity for *E. coli* was the highest and that for MRSA was the lowest among the four bacteria tested. © 2002 Wiley Periodicals, Inc. J Appl Polym Sci 86: 2977–2983, 2002

Key words: irradiation; polyesters; modification

INTRODUCTION

Poly(ethylene terephthalate) (PET) is frequently used to make prosthetic parts such as artificial vascular, laryngeal, and esophageal prostheses and sutures.[1] Bacterial infection often occurs when biomedical materials are used. This infection may be mild or asymptomatic and can be found only after the sudden death of the patient. Methicillin-resistant *Staphylococcus aureus* of an acute abscess infection culture[2] and *Pseudomonas aeruginosa* are common infectious bacteria found in hospitals. Entrohemorrhagic *Escherichid coli* O157:H7 is a pathogenic infectious bacterium found in recent years. The symptoms range from mild to serious hemorrhages and sometimes may result in hemolytic uremic syndrome, thrombotic thrombocytopenic purpura, and other complexes.[3,4]

Functional groups such as carboxylic acid, sulfonic acid, amide, amine, acrylate, pyrrolidone, and glycol groups are introduced into polymeric materials via chemical,[5] blending,[6] γ-ray,[7–9] plasma,[10] ozone,[11] UV,[12] and laser[13] methods. As one of the most effective means of modifying polymers, γ-rays can rapidly create uniform active radical sites on a polymer matrix. There are two types of irradiation grafting: simultaneous irradiation and preirradiation. Preirradiation forms less homopolymer.[14] The addition of metal salts can increase the reaction rate and suppress the formation of a homopolymer.[15]

The surface modification of polymers becomes important when polymeric materials come into contact with physiological components such as blood and living tissues. Chitosan (CS) and chitooligosaccharide (COS) are natural biocompatible and cationic polysaccharides. When sticking to the bacterial cell wall,[16] CS can suppress the metabolism of the bacteria. CS and its derivatives have functional groups, including hydroxyl, amine, and amide groups, and their modification (blending or grafting) is generally performed in powder, film, or solution forms.[17–19]

The antimicrobial activity of CS and its derivatives has received considerable attention recently. The main reason for choosing COS for this study is that COS can readily dissolve in water, unlike CS, which needs to be dissolved in an aqueous acetic acid solution. Therefore, the processability of COS is less cumbersome than that of CS. In addition, there would be no wasted acetic acid with COS. Furthermore, COS also shows antibacterial activity.

In this study, three types of polyester fibers were surface-modified to improve their performances in biomedical applications. The grafting adopted the preirradiation of ^{60}Co-γ-rays to reduce the formation of homopolymers. After preirradiation, the fibers were immediately reacted with the monomer to reduce the decay of surface peroxide. Metal salts were added to suppress the formation of homopolymers. Acrylic acid (AA) and *N*-vinylformamide (NVF) were grafted onto the fiber surface. Amino groups were obtained by the

Correspondence to: M.-C. Yang (myang@tx.ntust.edu.tw).

Journal of Applied Polymer Science, Vol. 86, 2977–2983 (2002)

Reaction Scheme I

$$\text{R-COOH} + \text{HO-Chitosan} \xrightarrow{1\text{N HCl}} \text{R-COO-Chitosan}$$

or

$$\text{R-COOH} + \text{HO-Chitooligosaccharide} \xrightarrow{1\text{N HCl}} \text{R-COO-Chitooligosaccharide}$$

where R = PET, PMPT, or PCHT

Reaction Scheme II

$$\text{R-NHCOH} \xrightarrow[\Delta]{1\text{N HCl}} \text{R-NH}_2$$

$$\text{R-NH}_2 + \text{OHC-(CH}_2)_3\text{-CHO} \longrightarrow \text{R-N=CH-(CH}_2)_3\text{-CHO}$$

$$\text{R-N=CH-(CH}_2)_3\text{-CHO} + \text{H}_2\text{N-Chitosan} \longrightarrow \text{R-N=CH-(CH}_2)_3\text{-CH=N-Chitosan}$$

or

$$\text{R-N=CH-(CH}_2)_3\text{-CHO} + \text{H}_2\text{N-Chitooligosaccharide} \longrightarrow \text{R-N=CH-(CH}_2)_3\text{-CH=N-Chitooligosaccharide}$$

Figure 1 Reaction schemes for the grafting of CS and COS onto the fiber surfaces.

hydrolysis of NVF. CS or COS was s then grafted to the NH_2-grafted fibers by a reaction with glutaraldehyde. Alternatively, CS or COS was grafted to AA-grafted fibers via esterification. These resulting fibers were tested for their antibacterial activity with four strains of pathogenic bacteria.

EXPERIMENTAL

Materials

Three polyester fibers—PET, poly(2-methyl-1,3-propylene terephthalate-*co*-ethylene terephthalate) (PMPT; 5%/95%), and poly(1,4-cyclohexylene terephthalate-*co*-ethylene terephthalate) (PCHT; 40%/60%)—were obtained from Far Eastern Textile Co., Ltd. (Hsinchu, Taiwan). These fibers were treated by Soxhlet extraction with methanol for 24 h for the removal of sizing and grease. CS (molecular weight $\sim 1.6 \times 10^5$, degree of deacetylation $\sim$ 75%) was obtained from China Textile Institute (Taipei, Taiwan), and COS (molecular weight = 1170) was obtained from Shin Era Technology Co. (Taipei, Taiwan). AA (99%) was purchased from Ferak Laborat GmbH (Berlin), and NVF was purchased from Aldrich Chemical Co. (Milwaukee, WI).

Postpolymerization

Fiber specimens were evacuated in plastic bags and preirradiated by ^{60}Co γ-rays (Institute of Nuclear Energy Research, Taoyuan, Taiwan) at a total irradiation rate of 10 kGy (dose rate = 7.8 kGy/h). Irradiated fibers were placed in 10 wt % AA or NVF aqueous solutions containing 0.2*M* H_2SO_4 and 0.001*M* $FeSO_4$. The reaction was carried out under nitrogen at 25, 50, or 75°C for 4 h. The resulting fibers were washed with double-distilled water three times in a 250-mL flask and then soaked in double-distilled water at 75°C for 24 h by the frequent replacement of water for the removal of homopolymers from the fiber surface. The hydrolysis of NVF-grafted fibers was carried out in 1*M* HCl at 55, 65, or 75°C for a specified period. This converted the amide groups of poly(*N*-vinylformamide) into amine groups.[20] The fibers were then washed as previously mentioned.

CS and COS grafting

For the grafting of CS or COS onto carboxyl-bearing fibers, the fibers were placed in 0.25 mg/mL CS in 5 m*M* acetic acid and 1*M* HCl or in 0.25 mg/mL COS in 1*M* HCl and were reacted at 60°C for 10 min. Afterward, the fibers were washed with a phosphate buffer solution (PBS) and double-distilled water three times. The reaction is given as scheme I in Figure 1.

For the grafting of CS or COS onto amino-bearing fibers, the fibers were first treated with 0.2*M* glutaraldehyde at 25°C for 30 min and were washed three times with PBS and double-distilled water; then, the fiber was dried in an oven at 65°C. These glutaraldehyde-treated fibers were then placed in a 0.25 mg/mL CS solution in 5 m*M* acetic acid or in 0.25 mg/mL COS in PBS (pH 7.4) and were reacted at 4°C for 16 h. Afterward, the fibers were washed with PBS and double-distilled water three times. The reaction is given as scheme II in Figure 1.

TABLE I
Clinical Sources of Bacteria Used in This Study

Bacterium strain	Source
Gram-positive bacteria	
MRSA	Acute abscess infection culture
S. aureus-2	Wound infection culture due to suture
Gram-negative bacteria	
E. coli O-157:H7	ATCC 43894
P. aeruginosa	ATCC10145

Antibacterial activity

Table I lists the four strains of bacteria used in this work. Frozen preserved stock was thawed at room temperature, and then 0.1 mL was pipetted and streaked into a quadrant on a sheep-blood agar plate (Difco Laboratories, Detroit, MI) and cultured at 37°C overnight. Afterward, a single colony was scraped with a loop and swabbed onto a 15°-slant medium (10 mL of nutrient agar) and was incubated at 37°C. After 18–24 h of culturing, 20 mL of PBS was added (72 mL of 0.2*M* Na_2HPO_4 was mixed with 28 mL of 0.2*M* NaH_2PO_4, and 0.5 g of NaCl and 2 g/L Tween 80 were added; this brought the total volume to 1000 mL). After mixing, 1 mL of the solution was moved into 9 mL of nutrient broth (concentration = 8 g/L) and mixed with a vortex mixer. The solution was then diluted with PBS to 1. $5 \pm 0.3 \times 10^5$ cell/mL and was placed in flasks (six samples, with 0.4 g per sample for each group). After incubation at 37°C for 0–18 h, 20 mL of PBS was added, and the mixture was stirred for 30 s. Consecutive dilute solutions were prepared by 1 mL of the previous solution being mixed with 9 mL of PBS. From this solution, 1 mL was transferred to a 50-mL centrifugal tube, mixed with 15 mL of nutrient agar (at 45°C), poured into a 9-cm plate, allowed to cool, and incubated at 37°C for 18–24 h. The number of surviving bacteria was then counted.

Analysis

Characterization of the preirradiated fiber

Twenty fibers were randomly selected and conditioned at 20°C and 65% relative humidity for 24 h. The fineness, strength, and elongation were measured according to ASTM Standard D 3822-1993 with two testing instruments (a Vibroskop single-fiber tensile tester and a Vivro dyn single-fiber linear density tester, Lenzing Technik, Lenzing, Austria).

Determination of the surface densities of carboxyl and amino groups

The surface density of carboxyl groups was measured from the uptake of a basic dye. The fiber was treated in 0.1 mg/mL toluidine blue O (the pH was adjusted with 0.1 m*M* NaOH to 10) at 30°C for 5 h. Afterward, the fiber was rinsed with double-distilled water, which was followed by 0.1 m*M* NaOH for the removal of the adsorbed dye. The desorption of the complexed dye was performed with a 50 vol % acetic acid solution. The surface density of the carboxyl groups was then determined from the absorbance[21] at 633 nm with a spectrophotometer (UV 3101 PC, Shimadzu, Tokyo).

The surface density of amino groups was measured from the uptake of an acidic dye. The fiber was treated in 0.1 mg/mL acid orange 7 (the pH was adjusted with 0.1 m*M* HCl to 3) at 30°C for 5 h. Afterward, the fiber was rinsed with double-distilled water, which was followed by 0.1 m*M* HCl for the removal of the adsorbed dye. The desorption of the complexed dye was performed with a 0.1 m*M* NaOH solution. The surface density of the amino groups was then determined from the absorbance at 485 nm.

Determination of the surface densities of CS and COS

The surface densities of CS and COS were measured from the uptake of an acidic dye. The fiber was treated in 0.1 mg/mL acid orange 7 (the pH was adjusted with 0.1 m*M* HCl to 3) at 30°C for 5 h. Afterward, the fiber was rinsed with double-distilled water, which was followed by 0.1 m*M* HCl for the removal of the adsorbed dye. The desorption of the complexed dye was performed with a 0.1 m*M* NaOH solution. The surface densities of CS and COS were then determined from the absorbance at 485 nm.

RESULTS AND DISCUSSION

Surface grafting of AA and NVF

Because of the high crystallinity and hydrophobicity and the lack of chemically active side groups, polyester fibers were ionized with γ-ray irradiation. The irradiated fibers were then reacted with either AA or *NVF*.

The grafting of AA was affected by both the temperature and substrate polymer, as shown in Figure 2. The surface density of carboxylic groups was higher for grafting at 50°C than for grafting at either 25 or 75°C. The higher grafting ratio at 50°C can be attributed to two factors: polymerization and free-radical decaying. A higher temperature is advantageous for the polymerization reaction, whereas a lower temperature is prevents the decay of the radicals. In general, free radicals in crystalline regions are more stable than those in amorphous regions, which migrate to the crystallite surfaces in time and then work efficiently for grafting.[22]

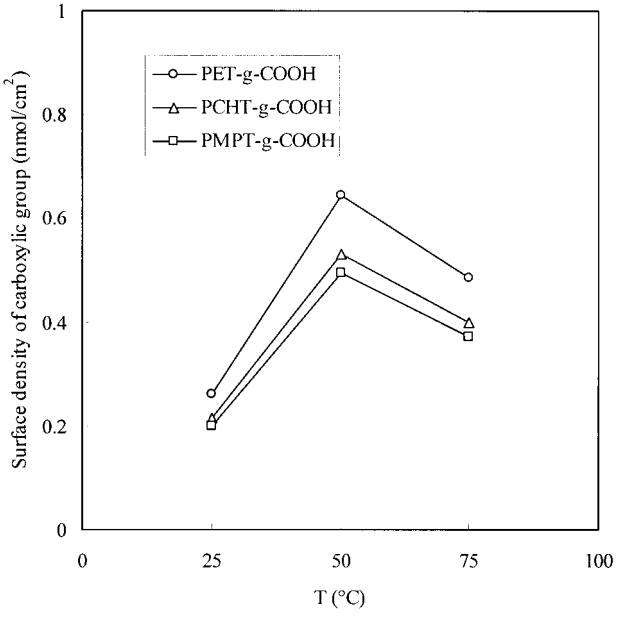

Figure 2 Amount of carboxylic groups introduced to the fiber at 25, 50, and 75°C for 4 h.

From Figure 2, it is obvious that PET had more carboxylic groups grafted than PCHT and PMPT. For polymers with double bonds, the elimination of free radicals is more effective than alkyl radicals.[23] When the polyester was irradiated with γ-rays, free radicals formed in both the crystalline and amorphous region. With longer alkyl chains, the amorphous regions would be partially crosslinked with one another or with adjacent double bonds. This would reduce the grafting effectiveness.[22] Because the side chain of PMPT had stereohindrance and PCHT had a cyclohexylene structure that could facilitate the migration of free radicals, these factors caused the grafting density of PMPT and PCHT surfaces to be lower than that of the PET surface. After γ-irradiation, the surface densities of peroxide were 0.85, 0.75, and 0.70 nmol/cm^2 for PET, PCHT, and PMPT, respectively. Polymeric peroxides introduced onto the irradiated fibers were determined spectrophotometrically by the iodide method.[24] Because the surface density of peroxide on PET surfaces is higher than those on PCHT and PMPT surfaces, more reactive sites were provided for AA.

The grafting of NVF showed a trend similar to that for AA, as shown in Figure 3. The surface density of NVF was determined indirectly by hydrolysis of the amide groups into amine groups, with 100% conversion assumed. The grafting density was higher for PET and at 50°C. However, the surface density of the grafted amino group was about one magnitude of order lower than that of AA. This agrees with the results of an earlier report.[21]

The hydrolysis of amide groups by HCl depends on the treatment temperature and time, as depicted in Figures 4–6. NVF was grafted at 50°C and hydrolyzed with 1 *N* HCl. As shown in Figure 4, the hydrolysis of NVF-grafted PET was completed in about 4–6 h with hydrolysis at 65 and 75°C, whereas it took more than 11 h to be completed at 55°C. As shown in Figures 5 and 6, similar trends were observed for both PMPT and PCHT, although their grafting densities were lower than that of PET.

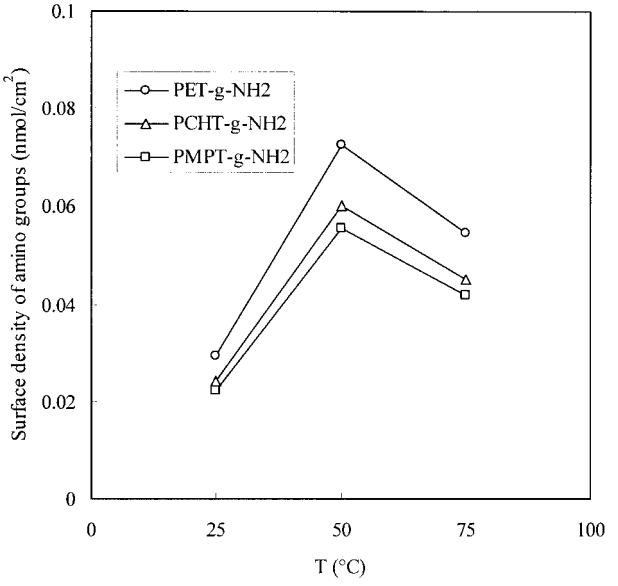

Figure 3 Effect of the temperature on the amount of NVF grafted onto the surfaces of PET, PMET, and PCHT. The NVF-grafted fiber was hydrolyzed with 1 *N* HCl at 75°C for 4 h for the conversion of the amide groups into amino groups.

The mechanical properties of the fibers were affected by the grafting, as shown in Table II. In general,

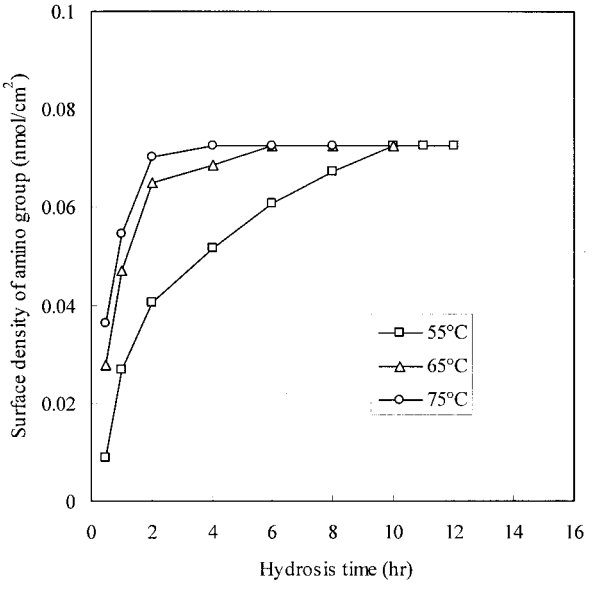

Figure 4 Effect of the temperature on the hydrolysis of NVF-grafted PET fibers.

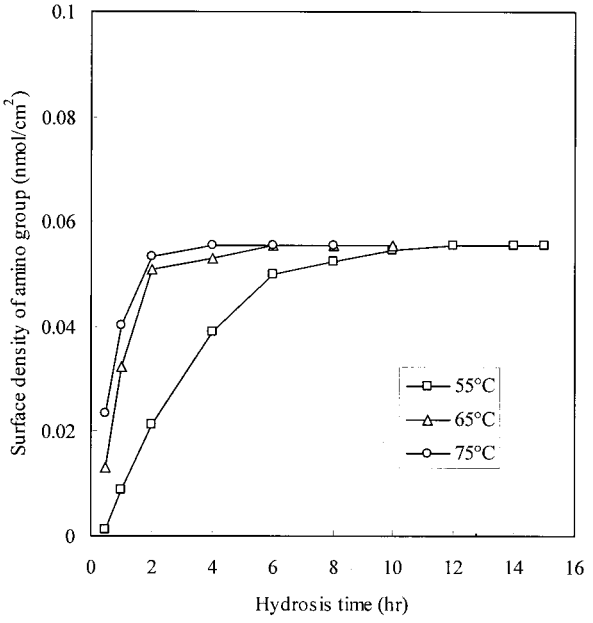

Figure 5 Effect of the temperature on the hydrolysis of NVF-grafted PMPT fibers.

the strength and elongation were reduced by the treatment. In particular, because of the extra hydrolysis, PET-*g*-NH_2 showed lower strength and less elongation than PET-*g*-COOH. The other two substrates, PMPT and PCHT, exhibited the same trend. The elongation of PET was reduced by 15% for PET, and the elongation of PMPT and PCHT changed little. Because of the cyclohexylene structure, PCHT lost more mechanical strength than the other two substrates, although there was only a 10% decrease in strength.

TABLE II
Mechanical Properties of Fibers Before and After Preirradiated Grafting

Material	Diameter (μm)	Strength (MPa)	Elongation (%)
PET	18.2 ± 6.6	655 ± 72	37.0 ± 4.6
PET-*g*-COOH	—	638 ± 66	33.6 ± 4.7
PET-*g*-NH_2	—	609 ± 71	31.9 ± 4.5
PMPT	17.0 ± 7.5	659 ± 104	35.6 ± 3.9
PMPT-*g*-COOH	—	644 ± 93	35.2 ± 6.8
PMPT-*g*-NH_2	—	633 ± 89	34.4 ± 6.1
PCHT	17.2 ± 7.3	583 ± 145	34.7 ± 5.8
PCHT-*g*-COOH	—	548 ± 165	34.6 ± 5.4
PCHT-*g*-NH_2	—	526 ± 168	34.0 ± 5.4

Grafting of CS and COS

The grafting densities of CS and COS are shown in Figure 7, in which the amounts of CS and COS are represented by the amino groups. In this study, CS and COS were grafted either via esterification with carboxylic groups or via the formation of imine linkages with glutaraldehyde, as described by the reaction schemes in Figure 1. Because CS and COS are polymers, there is more than one amino group per molecule. Therefore, the surface densities of CS and COS should be less than those of the amino groups shown in Figure 7. However, the grafting may react with more than one amino group for each molecule (in the case of imine formation), and as only unreacted amino groups can be detected, the actual number of molecules grafted remains uncertain. This plot can only show the trend of the grafting.

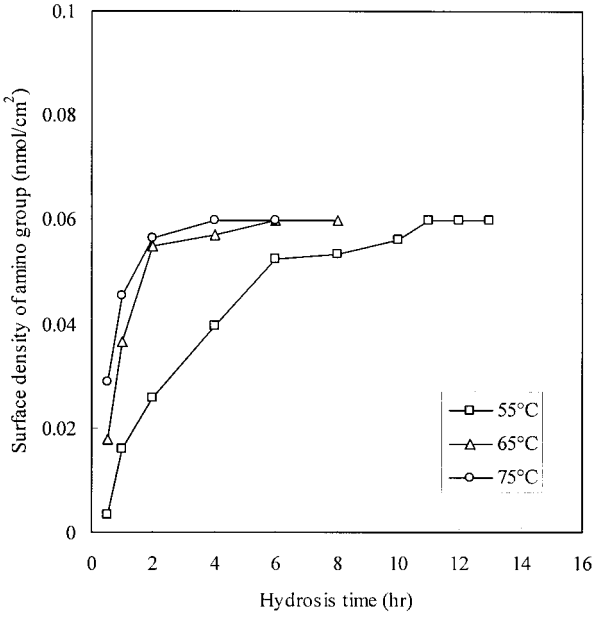

Figure 6 Effect of the temperature on the hydrolysis of NVF-grafted PCHT fibers.

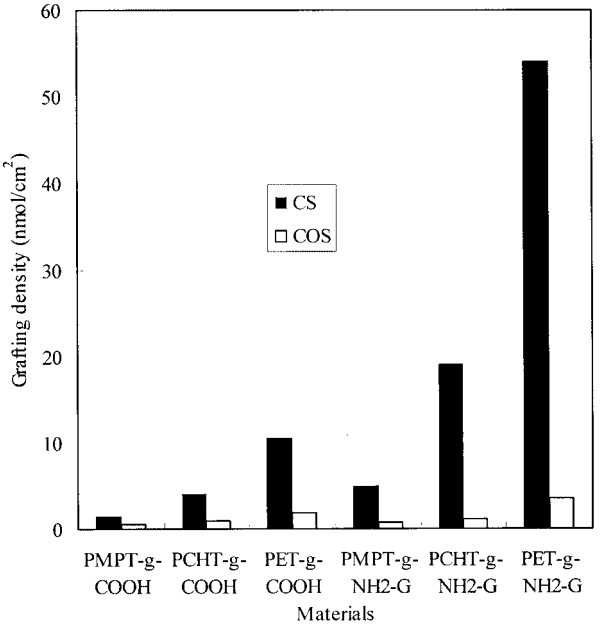

Figure 7 Surface density of CS and COS grafting to PET-*g*-COOH and PET-*g*-NH_2.

As expected, PET can attach to more CS or COS than the other two polymers because of its higher surface density. However, the grafting efficiency shows that esterification produced fewer amino groups than imine formation, even though the surface density of COOH was about 10 times higher than that of NH_2. This suggests that to graft onto the CS (or COS) molecule would require more COOH groups than NH_2 groups. This means that the grafting efficiency of esterification is lower than that of imine formation.

Antibacterial activities

Table I lists the four bacterial strains used in this study. Methicillin-resistant *S. aureus*-1 (MRSA) was taken from a clinical acute abscess infection culture, and *S. aureus*-2 was taken from a clinical wound infection culture. Because CS and its derivatives are frequently used for wound healing, their antibacterial activity is worthy of study. The other two strains, *E. coli* O-157:H7 and *P. aeruginosa*, are also pathogenic.

CS and COS carry positively charged NH_2 groups and can bind to the negatively charged cell walls of bacteria, so biosynthesis and energy transport through the cell wall would be hindered.[25] Eventually, the bacteria would be killed.

The antibacterial activity was investigated with CS- and COS-grafted PET fibers because of the higher surface density of NH_2. The initial inoculation was $1.5 \pm 0.3 \times 10^5$ CFU/mL.

Figure 8 shows the survival of MRSA with respect to the exposure time to CS-grafted (or COS-grafted) PET fibers or untreated PET fibers. For untreated PET, the concentration of MRSA grew to 3.16×10^7 CFU/mL after incubation at 37°C for 18 h. For those fibers grafted with either CS or COS, the concentration of MRSA changed little for the first 4 h and then began to decrease rapidly, and no bacteria could be detected after incubation at 37°C for 18 h. Among the four modified fibers, PET-NH_2-G-CS had the highest antibacterial activity, and PET-COO-COS had the lowest. This order agrees with the surface density of NH_2 groups shown in Figure 7. Similar results were observed for *S. aureus*-2, *E. coli*, and *P. aeruginosa*.

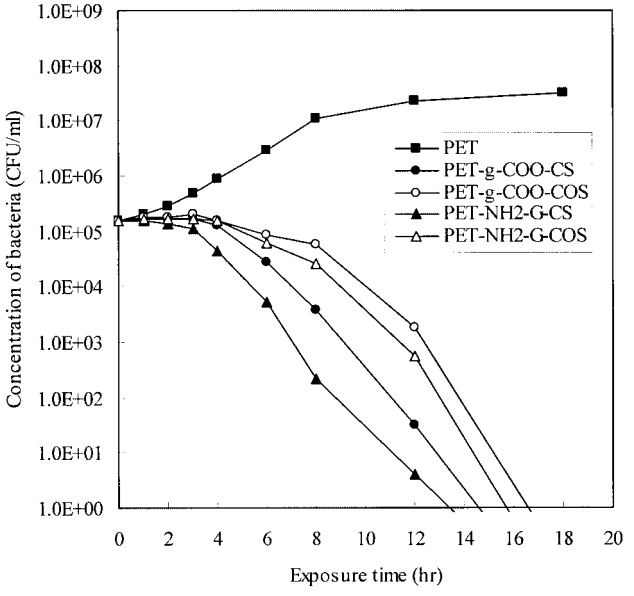

Figure 8 Effect of CS- and COS-grafted PET fiber surfaces on the variation of the survival ratio of MRSA with the exposure time.

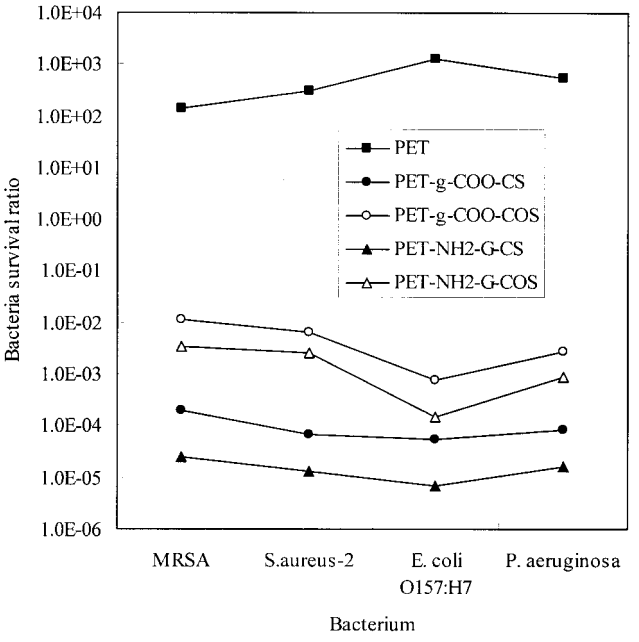

Figure 9 Comparison of the antibacterial activities of CS- and COS-grafted PET fibers for four pathogenic bacteria.

Figure 9 compares the antibacterial activity of these four CS- or COS-grafted PET fibers according to the survival ratios after incubation at 37°C for 12 h. We can conclude that these four grafted fibers can suppress the growth of all four bacteria used in this study and that the antibacterial activity increases with the surface density of NH_2 groups.

A previous study showed that the antibacterial activity of CS (or COS) is lower for *S. aureus* than for *E. coli* and *P. aeruginosa*.[26] Figure 9 shows that the antibacterial activity of CS (and COS) is as follows: *E. coli* > *P aeruginosa* > *S. aureus*-2 > MRSA. The extracellular capsule of MRSA makes it more hydrophobic than capsuleless *S. aureus*-2. A bacterium with an extracellular capsule carries fewer negative charges and is less prone to be adsorbed onto a positively charged fiber surface.[27] This makes MRSA less interactive with CS-grafted (or COS-grafted) PET than *S. aureus*-2. Gram-negative *E. coli* and *P. aeruginosa* have flagella on the structures external to the cell wall and, therefore, have mobility. *P. aeruginosa* has fewer flagella than *E. coli* and so is less mobile than *E. coli*. Furthermore, *E. coli*

has fimbriae, which make the bacterium more adsorbable. Therefore, the grafting of CS and COS is most antibacterial to *E. coli*.

CONCLUSIONS

Polyester fibers can be grafted with AA or NVF by preirradiation with γ-rays. By acid hydrolysis, amide groups on the fiber surface can be converted into amino groups. CS and COS can then be grafted to modified polyester surfaces by either esterification or imine formation. The highest surface density of amino groups can be achieved by imine formation between CS and glutaraldehyde-treated PET-*g*-NH_2.

CS- and COS-grafted polyesters show antibacterial activity for MRSA, *S. aureus*-2, *E. coli*, and *P. aeruginosa*. The antibacterial activity increases with the surface density of amino groups. Furthermore, the antibacterial activity for *E. coli* is higher than that for the other three bacteria, whereas the antibacterial activity for MRSA is the lowest.

References

1. An, A. H.; Friedman, R. J. J Microbiol Methods 1997, 30, 443.
2. Koneman, E. W.; Allen, S. D.; Janda, W. M.; Sohreckenberger, P. C.; Winn, W. C. Color Atlas and Textbook of Diagnostic Microbiology; Lippincott: New York, 1997; p 833.
3. Karmali, M. A.; Petric, M.; Lin, C. J Infect Dis 1985, 151, 775.
4. Karmali, M. A.; Steele, B. T.; Petric, M. Lancet 1983, 1, 619.
5. Karakisla, M.; Sacak, M. J Appl Polym Sci 1998, 70, 1701.
6. Shimizu, T.; Nagara, A.; Kaji, A.; Higashiura, S.; Ohguchi, M. J Appl Polym Sci 2000, 78, 392.
7. Sundardi, F. Polymer 1979, 20, 1522.
8. Rebenfeld, Y. L.; Weigmann, H. D. Text Res J 1978, 22, 125.
9. Park, J. S.; Kim, J. H.; Nho, Y. C.; Kwon, O. H. J Appl Polym Sci 1998, 69, 2213.
10. Ramires, P. A. J Biomed Mater Res 2000, 51, 535.
11. Fujimoto, K.; Takebayashi, Y.; Inoue, H.; Ikada, Y. J Polym Sci Part A: Polym Chem 1993, 31, 1035.
12. Uchida, E.; Uyama, Y.; Ikada, Y. J Appl Polym Sci 1993, 47, 417.
13. Dadsetan, M.; Mizadeh, H.; Sharifi, N.; Sanjani, N. S.; Salehian, P. J Biomed Mater 2001, 54, 540.
14. Ishigaki, I.; Sugo, T.; Takayama, T.; Okada, T.; Kamoto, J. O.; Machi, S. J Appl Polym Sci 1993, 27, 1043.
15. Dworjanyn, P. A.; Garnett, J. L.; Kahn, M. A.; Maojun, X.; Qian, M. P.; Nho, Y. C. Radiat Phys Chem 1993, 42, 31.
16. Kim, C. H.; Choi, J. W.; Chun, H. J.; Choi, K. S. Polym Bull 1997, 38, 387.
17. Pedram, M. Y.; Lagos, A. Pure Appl Chem A 1995, 32, 1037.
18. Fang, Y.; Liu, S.; Hu, D.; Cui, Y.; Xue, M. Polym Bull 1999, 43, 387.
19. Shigeno, Y.; Kondo, K.; Takemoto, K. J Macromol Sci Chem 1982, 17, 571.
20. Sano, S.; Kato, K.; Ikeda, Y. Biomaterials 1993, 14, 817.
21. Kato, K.; Ikeda, Y. Biotechnol Bioeng 1996, 51, 581.
22. Mitomo, H.; Enôjji, T. Pure Appl Chem A 1995, 32, 429.
23. Reichmanis, E.; Frank, C. W.; O'Donnell, J. H. Irradiation of Polymeric Materials: Process, Mechanisms, and Application; American Chemical Society: Washington, DC, 1993; p 29.
24. Frew, J. E.; Jones, P.; Scholes, G. Anal Chim Acta 1983, 155, 139.
25. Ikeda, T.; Hirayama, H.; Yamaguchi, H.; Tazuke, S. Antimicrob Agents Chemother 1986, 30, 132.
26. Uchida, Y.; Izume, M.; Ohtakara, A. Chitin and Chitosan; Elsevier Applied Science: London, 1988; p 373.
27. Hu, S.-G.;. Jou, C.-H.; Yang, M.-C. J Appl Polym Sci, submitted.