Surface Characterization and Antibacterial Activity of Chitosan-Grafted Poly(ethylene terephthalate) Prepared by Plasma Glow Discharge

MAN WOO HUH,[1] INN-KYU KANG,[2] DU HYUN LEE,[2] WOO SIK KIM,[2] DONG HO LEE,[2] LEE SOON PARK,[2] KYUNG EUN MIN,[2] KWAN HO SEO[2]

[1] Department of Textile Engineering, Kyungil University, Kyungsan 712-701, South Korea

[2] Department of Polymer Science, Kyungpook National University, Taegu 702-701, South Korea

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ABSTRACT: Poly(ethylene terephthalate) (PET) texture was exposed to oxygen plasma glow discharge to produce peroxides on its surfaces. These peroxides were then used as catalysts for the polymerization of acrylic acid (AA) in order to prepare a PET introduced by a carboxylic acid group (PET-A). Chitosan and quaternized chitosan (QC) were then coupled with the carboxyl groups on the PET-A to obtain chitosan-grafted PET (PET-A-C) and QC-grafted PET (PET-A-QC), respectively. These surface-modified PETs were characterized by attenuated total reflection Fourier transform IR spectroscopy, electron spectroscopy for chemical analysis, and a contact angle goniometer. The amounts of AA, chitosan, and QC grafted on the PET surfaces as determined by the gravimetric method were about 6, 8, and 9 $\mu g/cm^2$, respectively. The antibacterial activity of the surface-modified PET textures was investigated using a shake flask method. After 6 h of shaking, the growth of bacteria was markedly inhibited by PET with ionically (86% in PET-A^--C^+) and covalently (75% in PET-A-C) grafted chitosan and with covalently grafted QC (83% in PET-A-QC). After the laundering the inhibition of the growth of the bacteria was maintained in the range of 48–58%, showing the fastness of the chitosan-grafted PET textures against laundering. © 2001 John Wiley & Sons, Inc. J Appl Polym Sci 81: 2769–2778, 2001

Key words: poly(ethylene terephthalate); plasma glow discharge; chitosan; antibacterial activity

INTRODUCTION

Recent studies were focused on the development of antibacterial surfaces to attain high functionality and high value products.[1,2] Poly(ethylene terephthlates) (PETs) are often used as basic materials in the textile and plastics industries. Accordingly, the improvement of the antibacterial properties of PETs is important for a wide range of industrial applications including cosmetics, clothing, shoes, bedding, and interior materials for automobiles.[3,4] In contrast, chitosan, which is a polysaccharide obtained by the deacetylation of chitin, is frequently used as a wound healing accelerator or antibacterial processing agent.[5] The bacteriostatic action of chitosan was demonstrated by Allen et al.[6] against organisms selected from common skin bacteria. They found that

Correspondence to: I.-Y. Kang.

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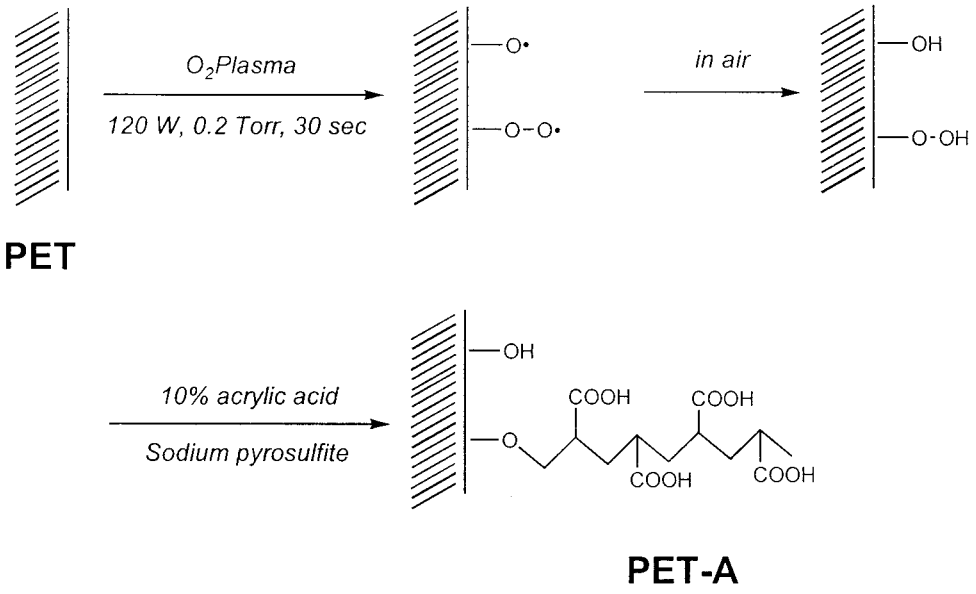


Scheme 1 Oxygen plasma treatment of PET and graft polymerization of acrylic acid (AA) on PET.

Staphylococcus epidermidis was completely inhibited by a 0.1% solution of chitosan, yet *S. aureus* and *P. aeruginosa* needed a 1% concentration. Shibasaki et al.[7] reported that the presence of low molecular weight chitosan in drinking water could inhibit the formation of plaque and cavities due to the inhibition of microbial growth.

Several approaches for generating functional groups on the surface of PETs were reported.[8,9] One approach was to hydrolyze PETs with an alkaline aqueous solution. Ito et al.[8] prepared insulin-immobilized PETs by the hydrolysis of PETs and the subsequent reaction with insulin. Another approach was to treat the surfaces with plasma glow discharge. Plasma glow discharge treatment is an effective method for introducing biological molecules to the surfaces of polymeric materials without causing any severe damage to their bulk properties.[9–12] Kim et al.[9] prepared PET coimmobilized with insulin and heparin using oxygen plasma glow discharge treatment followed by acrylic acid (AA) grafting and a subsequent reaction with insulin and heparin.

In this study PET surfaces were chemically treated with oxygen plasma glow discharge, which was followed by graft polymerization of AA. Chitosan or quaternized chitosan (QC) was then coupled to the carboxylic acid groups on the surfaces. Thereafter, the chitosan-immobilized PETs were characterized using attenuated total reflection Fourier transform IR (ATR-FTIR) spectroscopy, electron spectroscopy for chemical analysis (ESCA), and a contact angle goniometer. The growth inhibition of the bacteria on the surface-modified PETs was also examined.

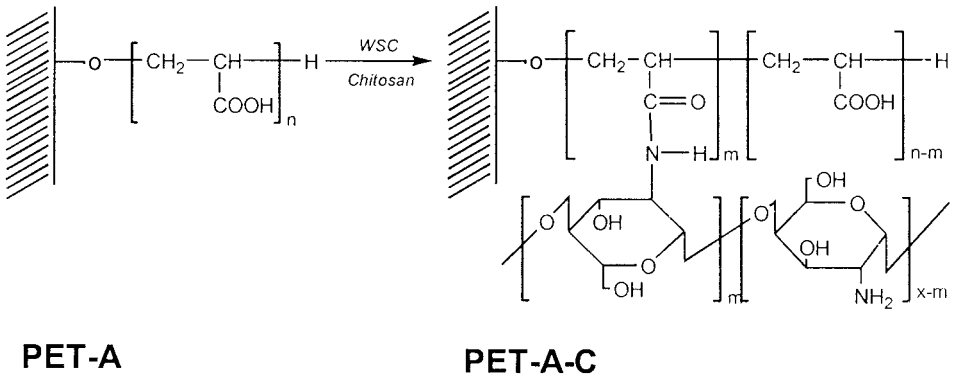
EXPERIMENTAL

Materials

The PET (woven texture and film) used in this study was kindly donated by the Kolon Company. The chitosan (≥85% degree of deacetylation, Sigma) was dissolved in a 1% (v/v) aqueous acetic acid solution, filtrated by a glass filter to remove the insoluble part, reprecipitated, and finally dried under reduced pressure before use. The bacteria used in this study were purchased from the Gene bank of the Institute of Life Sciences and Biotechnology in Korea (*S. aureus* ATCC 6538).

Plasma Glow Discharge Treatment and AA Grafting

The oxygen plasma treatment of the PET texture (4×4 cm^2) was carried out in accordance with a previous report.[9] The oxygen plasma treated PET was immersed in a 10 wt % AA aqueous solution, then sodium pyrosulfite was added as the reducing agent to obtain AA-grafted PETs (PET-A) as shown in Scheme 1. After a 24-h incubation the



Scheme 2 Schematic diagram showing the formation of chitosan-grafted poly(ethylene terephthalate) (PET-A-C).

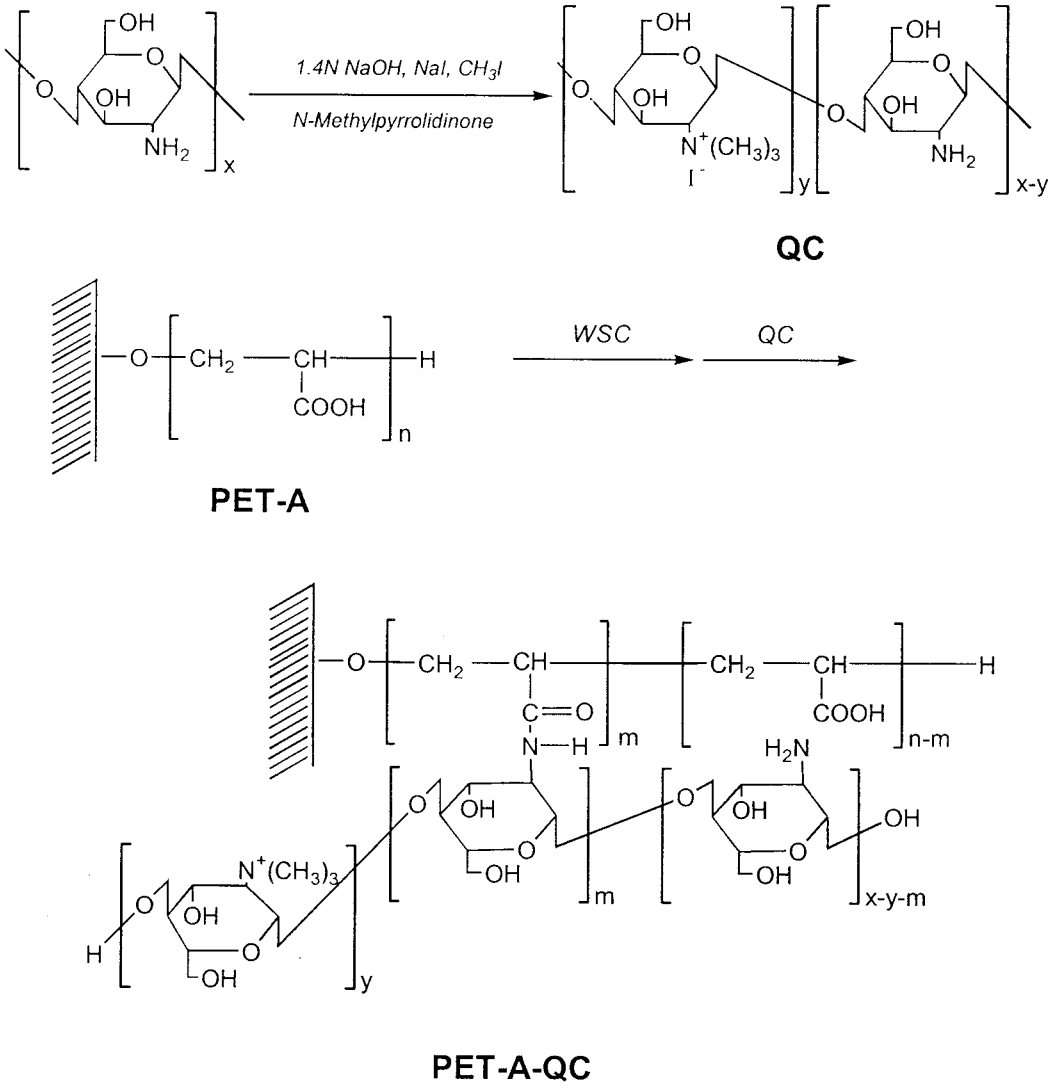
sample was taken and washed with a 0.1% Triton X-100 aqueous solution in an ultrasonic cleaner for 5 min to remove any free homopolymers that had formed. The sample was then rewashed using an ultrasonic cleaner filled with distilled water and dried under reduced pressure for 24 h at room temperature. The amount of AA grafted onto the PET surfaces was calculated by measuring the weight of the PET texture (1×2 cm^2) before and after the AA grafting using a microbalance (Sartorius XM 1000P).

Chitosan Grafting

The chitosan (5 g) was dissolved in a 2% (v/v) aqueous acetic acid solution (45 mL) and the solution was then filtrated by a glass filter to remove the insoluble part. The filtrate was poured into a 0.1*N* NaOH aqueous solution. The resulting precipitate was purified by washing with distilled water and dried under reduced pressure for 24 h. The molecular weight of the chitosan calculated using the Huggins equation was about 575,000. The purified chitosan (0.25 g) was dissolved in a mixed solution (50 mL, pH 4.7) of acetic acid and sodium acetate, and the pH of the chitosan solution was adjusted to 5.5 using a 1*N* NaOH aqueous solution. The immobilization of chitosan on the surfaces through ionic bonds was carried out by first dipping the PET-A in the chitosan solution for 8 h and then leaving it in a dry oven for 5 min at 120°C and another 5 min at 160°C. Through this process the ionically bound chitosans are physically anchored on the surface of the PETs, producing the PET-A$^-$-C$^+$ sample. In contrast, the immobilization of chitosan through covalent bonds was carried out as shown in Scheme 2. The PET-A was dipped in a 0.1% (w/v) 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (WSC) aqueous solution for 3 h at 4°C to activate the carboxyl groups on the surfaces, and it was subsequently transferred into the chitosan solution to obtain chitosan-grafted PET (PET-A-C). The amount of grafted chitosan was determined using the gravimetric method as described in the experiment for the AA grafting.

QC Grafting

The quaternization of chitosan was carried out according to the method[13,14] previously reported (Scheme 3). In brief, chitosan (5 g) was added to *N*-methyl-2-pyrrolidinone (250 mL) and suspended by stirring at room temperature for 12 h. The temperature of the suspension solution was then lowered to 4°C using ice water. A 1.4*N* NaOH aqueous solution (43 mL), sodium iodide (6 g), and methyl iodide (64 g) were added to this solution and it was kept at 36°C for 6 h while stirring. The reaction solution was then filtered using a mesh (100 mesh) to remove the insoluble part. The filtrate was precipitated into a large excess of acetone and filtrated using a filter paper. This process was repeated 5 times and the resulting product was dried under reduced pressure for 24 h. The PET-A (4×4 cm^2) was dipped into a sodium citrate buffer solution containing 0.1% (w/v) water-soluble carbodiimide at 4°C for 3 h to activate the carboxyl groups on the surfaces. The activated PET-A was then dipped into a QC solution at 4°C for 24 h to obtain QC-grafted PET (PET-A-QC). The amount of QC grafted on the PET was measured using the same method as described in the experiment for the AA grafting.



Scheme 3 Schematic diagram showing the quaterization of chitosans and their immobilization on PET-A.

Surface Characterization

The ATR-FTIR spectra of the surface-modified PETs were obtained using a Bruker spectrophotometer equipped with a KRS-5 reflection element. The surface-modified PETs were also analyzed with ESCA equipment (ESCALAB MKII, V. G. Scientific Co., East Grinstead, U.K.) equipped with Mg Kα at 1253.6 eV with a 100-W power at the anode. Spectra were taken at an angle of 55°. For the evaluation of the surface wettability the water contact angles of the surface-modified PETs were measured at room temperature using a contact angle goniometer[15] (model G-I, Erma Inc., Tokyo). A drop of water was placed on the air-side surface of the modified PETs at 25°C, and after 30 s the contact angle was measured. More than five measurements were carried out for a single sample and the values obtained were averaged.

Bacterial Strain and Culture

The *S. aureus* (ATCC 6538) was maintained on nutrient agar plates consisting of peptone, beef extract, sodium chloride, and agar at 4°C. Single colonies were then transferred to soy broth and incubated at 37°C for 10 h. The cell concentration was adjusted to 2×10^8 cells mL^{-1} by dilution with a phosphate-buffered solution (PBS), which was also used as the preserving solution for all the cell experiments.

Determination of Cultured Bacteria

A graded series of cell concentrations were prepared by diluting the preserving solution with PBS and measuring their absorbance at 500 nm. The relationship between the cell concentration and the absorbance at 500 nm was constructed for a calibration. The concentration of the cells grown in the flask was then calculated based on this calibration.

Antibacterial Activity of Chitosan-Grafted PETs

The antibacterial activity of the chitosan-grafted PETs was investigated using a shake flask method.[16] This method was developed by the Dow Corning Co. and is often used for the evaluation of the antimicrobial activity of textile products treated with nonreleased processing reagents. Monopotassium phosphate (34 g) was dissolved in deionized water (500 mL), and the pH of the phosphate aqueous solution was adjusted to 7.2 using a 4*N* NaOH aqueous solution. The PBS was sterilized using an autoclave (120°C, 20 min), diluted with distilled water (to 800 times), and then used for the shaking flask culture test. The preserving solution was diluted with PBS (to 1000 times). Next, 5 mL of the diluted cell solution was added to a triangle flask (200 mL) and mixed with PBS (70 mL). The final cell concentration was 1.5×10^4 cells mL^{-1}. One milliliter of the cell solution was taken from three different parts in the flask and diluted with PBS. One milliliter of the diluted cell solution was then seeded on an agar plate and incubated at 37°C for 24 h. The number in the colony (A) was counted by measuring the colony formed and compensating with the degree of cell dilution. The PET sample (1×1 cm^2) was added to the flask supplemented with the diluted cell solution and the mixture incubated in a shaking incubator (150 rpm, KMC 8480S, Vision Scientific Co.) for the requisite time. After incubation, 1 mL of the cell solution was extracted, seeded on an agar plate, and incubated at 37°C for 24 h. The number in the colony (B) was counted by measuring the colony formed and compensating with the degree of cell dilution. The inhibition of the cell growth was calculated using the following equation:

$$\text{growth inhibition of cell (\%)} = (A - B)/A \times 100$$

where A and B are the number in the colony before and after shaking, respectively. The experiment was repeated in quadruplicate and a mean value calculated.

Effect of Laundering on Antibacterial Activity

The laundering of the PET sample was carried out using a launder-o-meter (Matis Labomat Beaker Dyer BFA 9-16, Wener Matis AGCO Co.) that contained eight stainless steel test bottles (450-mL bottle volume). Each bottle contained soaps (5 g L^{-1}), sodium carbonate (2 g L^{-1}), distilled water (100 mL), and 10 stainless steel beads. The PET sample (1×1 cm^2) was placed in a test bottle and the laundering was carried out at 70 ± 2°C for 45 min. After laundering the sample was washed twice with distilled water (100 mL) for 1 min to remove any remaining washing solution. The sample was then dried under a thermal convection oven at 60°C for 30 min. The antibacterial activity of the laundered sample was examined using the same method as described in the experiment with the chitosan-grafted PETs.[17] The experiment was carried out 4 times and a mean value was calculated.

RESULTS AND DISCUSSION

Surface Characterization

The chitosan with over 85% deacetylation was used for the immobilization reaction on PET surfaces and subjected to the quaternization reaction. Domard et al. reported that the degree of quaternization of chitosan is proportional to the reaction time and reaches around 0.64 after 9 h when it is reacted with sodium iodide and methyl iodide.[18] In this study the quaternization of the chitosan was carried out for 6 h according to the method reported previously. The degree of quaternization of chitosan was therefore predicted to be about 0.4 when the calculation was based on the results of Domard et al.[18,19]

Figure 1 shows the ATR-FTIR spectra of the surface-modified PETs. In the preliminary experiment we was found that in PET the peak of the carboxylic acid groups in the grafted AA groups overlapped that of the ester groups. Therefore, in this study the PET-A was treated with methanolic sodium hydroxide to convert the carboxylic acid into sodium carboxylate.[20] As shown in spectrum b in Figure 1, a new peak appeared at around 1550 cm^{-1} that was based on carboxylate (COO^-), thereby suggesting the successful intro-

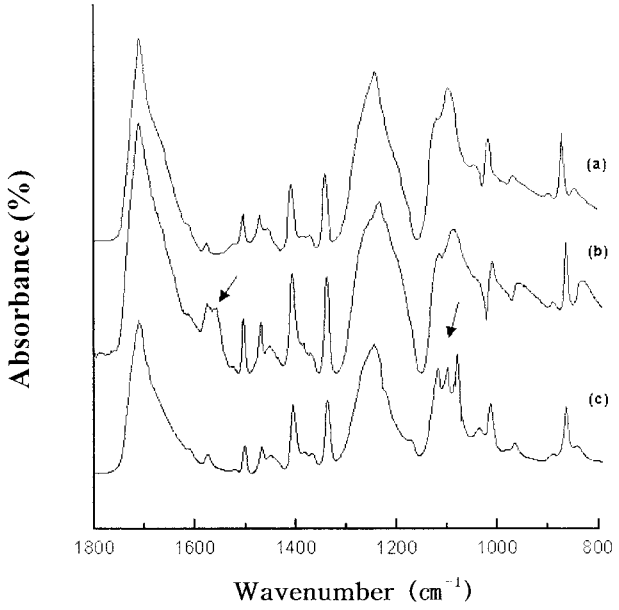


Figure 1 ATR-FTIR spectra of PET (spectrum a), PET-A treated with 4*N* NaOH aqueous solution (spectrum b), and PET-A-C (spectrum c).

duction of AA. In the spectrum of PET-A-C a new peak appeared at around 1100 cm^{-1}, which was possibly due to the ether linkage (C—O—C) of the grafted chitosan.

The changes in the chemical structure of the surface-modified PETs were investigated using ESCA.[21] Figure 2 shows ESCA survey scans of the PET (Fig. 2, spectrum a), PET-A (Fig. 2, spectrum b), and PET-A-C (Fig. 2, spectrum c) surfaces. As expected, the PET and PET-A surfaces showed two peaks corresponding to C1s (285-eV binding energy) and O1s (532-eV binding energy).[22] In the spectrum of PET-A-C an additional peak at 400-eV binding energy based on N1s was found, thereby indicating the successful grafting of chitosan onto the PET surfaces. The chemical compositions of the surface-modified PETs calculated from the ESCA survey scan spectra are shown in Table I. The oxygen content (27.1%) of the PET surface was increased by AA grafting (33.9% PET-A) due to the high oxygen content of the AA itself. In contrast, nitrogen

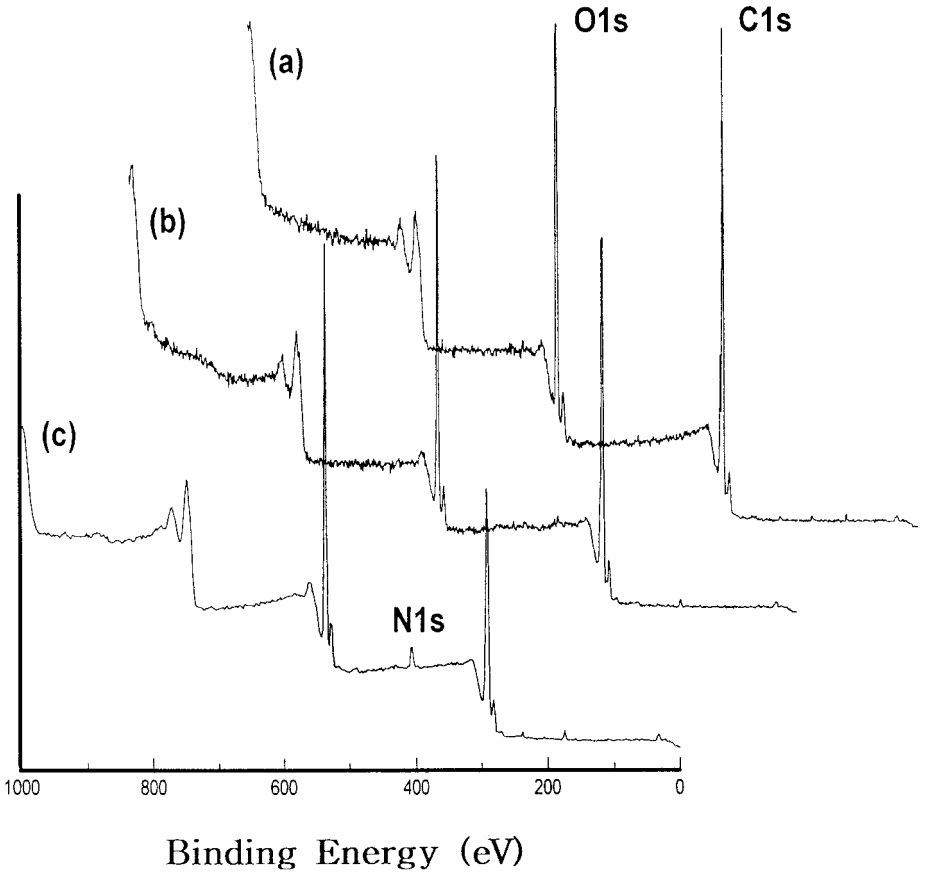


Figure 2 ESCA survey scan spectra of PET (spectrum a), PET-A (spectrum b), and PET-A-C (spectrum c).

Table I Elemental Composition of Surface-Modified PETs Calculated from ESCA Survey Scan Spectra

	Atomic Percentage		
Substrates	C	O	N
PET	72.9	27.1	—
PET-A	66.1	33.9	—
PET-A-C	63.9	32.4	3.7

The plasma conditions were 120 W, 0.2 Torr, and 30 s.

(3.7%) was found on the surface of PET-A-C, which was attributed to the amine groups attached to the C_2 carbon of the grafted chitosan. The C1s core-level spectra of the surface-modified PETs are shown in Figure 3. The pattern of the C1s core-level scan spectra of PET [Fig. 3(a)] was almost the same as that of PET-A [Fig. 3(b)]. This

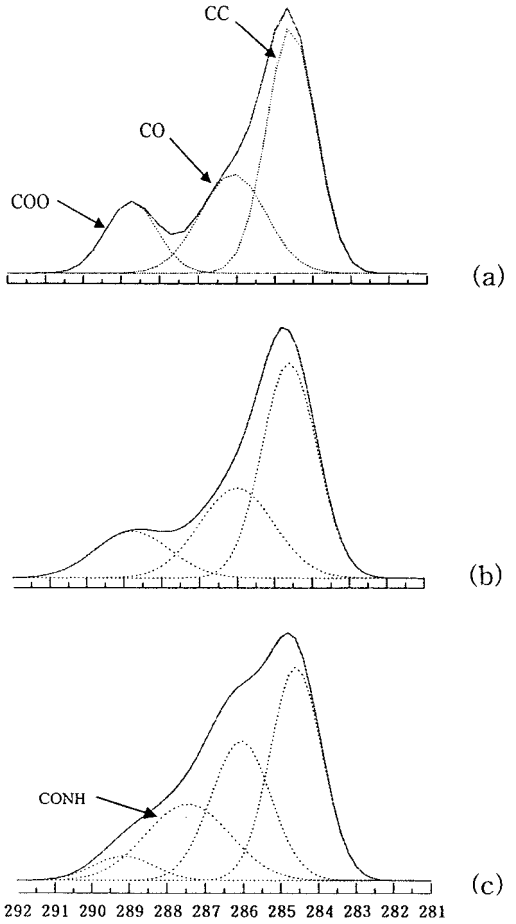


Figure 3 ESCA C1s core-level scan spectra of (a) PET, (b) PET-A, and (c) PET-A-C.

Table II Water Contact Angles of Surface-Modified PET Films

Substrates	Contact Angle[a] (°)
PET	72 ± 3
PET-A	29 ± 2
PET-A-C	30 ± 2
PET-A-QC	29 ± 2

[a] Measured by the sessile droplet method.

was because the binding energy of the C1s, based on the carboxylic acid groups grafted on the PET-A, overlapped that of the ester groups in the PET control.[9] In the chitosan-grafted PET (PET-A-C), however, the peak at the binding energy of 289 eV was largely decreased whereas a new peak appeared at around 288 eV. Accordingly, it would appear that a large amount of the carboxylic acid in the grafted AA reacted with the primary amine groups in the chitosan, resulting in the generation of amide bonds (—CONH—).

To compare the wettabilities of the surface-modified PETs, the water-contact angles of the surfaces were measured in air using the sessile droplet method. As shown in Table II, the water contact angle of PET (72°) in air was decreased with the introduction of the carboxylic acid groups (29°) and chitosan (30°). This indicates that the surfaces of PET became more wettable after surface modification.[9,23] The amount of functional groups on PET as determined by the gravimetric method is shown in Table III. The results showed that the amount of AA grafted on the PET surface was 6 $\mu g/cm^2$ (0.083 $\mu mol/cm^2$), and the amount of chitosan (8 $\mu g/cm^2$) coupled to the PET-A surface did not differ much from that of QC (9 $\mu g/cm^2$). Kim et al.[9] prepared the heparin-immobilized PETs by an oxygen plasma treatment followed by AA grafting and a subsequent

Table III Amount of Acrylic Acid (AA) and Chitosan (C, QC) Grafted on PETs

Substrate	AA ($\mu g/cm^2$)	C ($\mu g/cm^2$)	QC ($\mu g/cm^2$)
PET-A	6 ± 2	—	—
PET-A-C	—	8 ± 2	—
PET-A-QC	—	—	9 ± 2

The grafted amounts were measured by the gravimetric method.

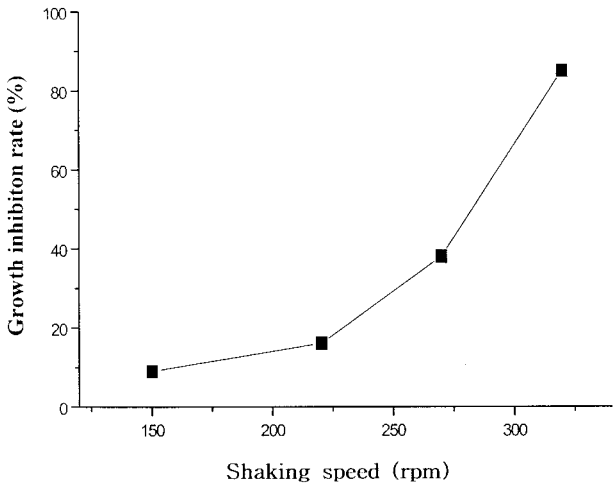


Figure 4 The effect of the shaking speed on bacteria growth inhibition in a flask.

reaction with heparins. The amount of heparins introduced, as determined by a dye interaction, was 1.23 $\mu g/cm^2$. Comparing this study with the previous one,[9] the amount of chitosan (8 $\mu g/cm^2$) immobilized on the PET-A was larger than that of heparin (1.23 $\mu g/cm^2$) immobilized on the same substrate. This result may have originated from the fact that the content of primary amine groups, which can react with carboxyl groups on the PET surfaces, in the chitosan was larger than that in heparin.[24]

Antibacterial Activity

A shaking flask method was employed to estimate the antibacterial activity of chitosan-grafted PETs. The proliferation of bacteria in a PBS solution can be influenced by the shaking speed of the cell suspension. Figure 4 shows the effect of the shaking speed of the bacteria suspension on growth inhibition. The growth inhibition of the cells in the PBS decreased with an increase in the shaking speed. The growth inhibition was about 8% when shaking at 150 rpm, as shown in Figure 4. Therefore, the shaking speed of the flask was fixed at 150 rpm for the antibacterial activity test. Figure 5 represents the growth inhibition of bacteria on the surface-modified PETs after 1- and 6-h shaking. The growth inhibition of bacteria after a 1-h shaking was lower than that after a 6-h shaking, irrespective of the kind of substrate. The growth inhibition (8%) of the PET control after a 1-h shaking was almost the same as that of PET-A (9%). This indicated that the growth of *S. aureus* was not much influenced by contact with PETs or AA-grafted PETs. However, the growth of bacteria was significantly inhibited by contact with chitosan-grafted PETs (62% in PET-A^--C^+, 39% in PET-A-C, 59% in PET-A-QC). After 6 h of shaking the growth of bacteria was markedly inhibited by PET with ionically (86%) and covalently (75%) grafted chitosan and covalently grafted QC (83%). The PET-A^--C^+ (86%) showed higher antibacterial activity than PET-A-C (75%). The high growth inhibition of the cells by PET-A^--C^+ may have originated from the easy release of chitosan from the matrices. In PET-A^--C^+ the chitosans were ionically bound to the carboxyl groups on the surfaces. As a result, these chitosans could be released from the PET surface during shaking in the flask, thereby inhibiting the bacterial growth in the medium. The high growth inhibition by PET-A-QC seems to be attributed to the quaternary ammonium ions of the grafted chitosan. Imazato et al.[25] reported that immobilization of the bactericide in dental resin composite could be achieved by 12-methacryloyl dodecylpyridinium bromide. Several studies[26–28] reported the antibacterial activity of immobilized quaternary ammonium as a contact antimicrobial, including *N*-alkyldimethylbenzylammonium chloride. Kanazawa et al.[29,30] also reported that immobilization of a polycationic biocide containing phosphonium salt on the surface of polypropylene film or cellulose showed an interesting bactericidal effect on *S. aureus* and *Escherichia coli*. The mechanism of the antibacterial activity of the immobilized agent was speculated to be disruption of cell membrane function by the active portion that is chemically bound to the carrier material.[31]

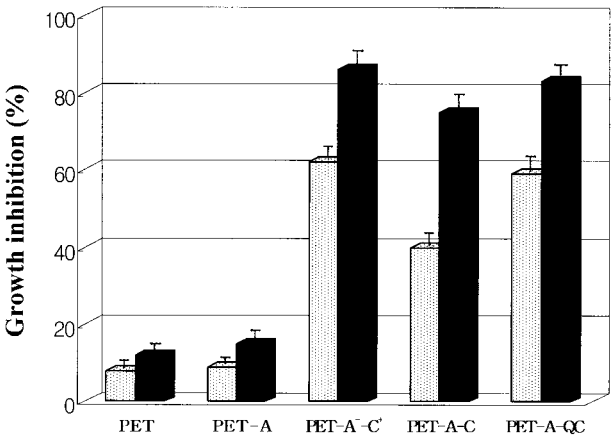


Figure 5 The bacteria growth inhibition by surface-modified PETs after (□) 1 and (■) 6 h of shaking.

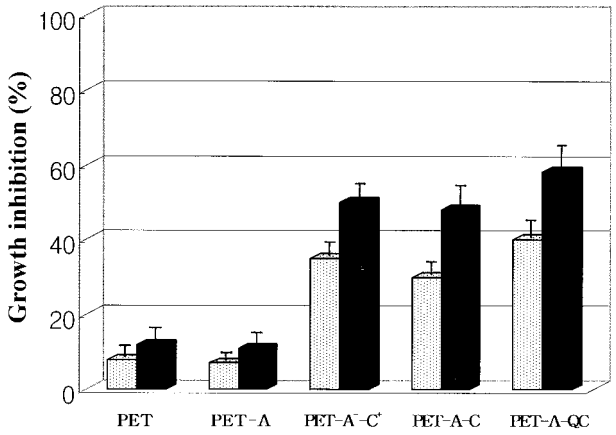


Figure 6 The bacteria growth inhibition by the laundered surface modified PETs after (□) 1 and (■) 6 h of shaking.

Laundering Effect on Antibacterial Activity

The growth inhibition of *S. aureus* on modified PETs before and after laundering was measured using the shaking flask method in order to evaluate the laundering effect on antibacterial activity, and the results are shown in Figure 6. The growth inhibition of *S. aureus* on the PET control and PET-A was below 10%, irrespective of the shaking time. Cell growth was slightly more suppressed after 6-h shaking than after 1 h, and these patterns were similar to those obtained without laundering (Fig. 5). After 6 h of shaking the PET-A^--C^+ and PET-A-C showed 50 and 48% cell growth inhibitions, respectively, while PET-A-QC showed a 58% cell growth inhibition. Shim et al.[17] synthesized copolymers of *N*,*N*′-dimethyl-*N*,*N*′-diallylammonium chloride and acrylamide and methyolated with hydroxyl groups of cellulose to produce antimicrobial agents. In their results, the cotton fabric finished by the methyolated copolymers showed 90% cell growth inhibition after laundering in anionic commericial detergent. As established from the results of the antibacterial activity obtained before (Fig. 5) and after (Fig. 6) laundering, the cell growth inhibition on PET-A^--C^+ decreased with laundering from 86 to 50%. In PET-A-QC the cell growth inhibition decreased from 83 to 58% with laundering. These results suggests that the parts of the chitosans that were bound either ionically or covalently to the PET surfaces were released into the washing medium during laundering.

CONCLUSION

A treatment of PET textures with oxygen plasma glow discharge followed by a graft polymerization of AA on the surfaces was used to prepare PET-A. Chitosan and QC were then ionically or covalently immobilized on PET-A. The AA grafting and chitosan immobilization on PET surfaces were identified using ATR-FTIR and ESCA. In the experiments of antibacterial activity using *S. aureus*, the chitosan-grafted PETs (PET-A^--C^+, PET-A-C, PET-A-QC) showed a high growth inhibition in the range of 75–86% and still maintained a 48–58% bacterial growth inhibition after laundering.

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