An Improvement on Water Absorbing and Permeating Properties: Heparin Immobilizing on Acrylic Acid-Grafted and Collagen/Chitosan-Immobilized Wound Dressing

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ABSTRACT: Various concentrations of heparin were employed in this study to immobilize on the acrylic acid-grafted and collagen/chitosan-immobilized polypropylene (PP-AAg-CCi) nonwoven fabric to improve the water absorbing and permeating properties. The immobilized heparin was verified by analyzing of the spectra of surface reflection infrared spectroscopy. It was found that the values of water absorbing and water diffusion coefficient for the PP-AAg-CCi sample immobilized with heparin (PP-AAg-CCHi) were significantly higher than those for the PP-AAg-CCi. The bacteria inhibition percentage and bacteria inhibition zone for the PP-AAg-CCHi were excellently. The pore and agent distribution for PP-AAg-CCi were examined with scanning electron microscope photographs. © 2008 Wiley Periodicals, Inc. J Appl Polym Sci 109: 1431–1438, 2008

Key words: composites; crosslinking; diffusion; FTIR; swelling

INTRODUCTION

Collagen, which emerges early during embryonic development and constructs the structural framework of most organs, is responsible for the functional integrity of such tissues as bones, cartilages, and skins. Quite rich in proteins accounting for about 30% of all proteins present in mammals,[1–4] collagen is found to contain good biocompatibility.[1] On the other hand, as is already well-known, chitosan, partially deacetylated from chitin, possesses good antibacterial activities, and cell adhesiveness[5] with high thermoplastic properties.[5–8] Yang et al.,[9] for instance, for dressing wounds, used chitosan to impregnate on the acrylic acids (AA) and *N*-isopropyl acrylamide bi-grafted polypropylene (PP) nonwoven fabrics, which had tremendous values of water vapor transmission rates as well as antibacteria. Min and coworkers[10] introduced the web of nano-fibers of electro-spinning collagen to be coated with native extracellular matrix for wound dressing and found that collagen nano-fibrous matrix was a very effective wound-healing accelerators in early-stage of wound healing. Some products derived from collagen and chitosan or the combined materials for wound healing have been developed in the recent years and some of them have been approved and are now commercially available.[11–13] Our previous study[14] showed that the water absorbing property and water diffusion coefficient of the AA-grafted and collagen/chitosan-immobilized PP nonwoven fabric decreased with the increase of chitosan contained in the collagen/chitosan immobilizing agent. Nevertheless, drawbacks still exist in the products derived from collagen or chitosan, such as the water absorbing and water permeating properties. For large open wounds, a durable dressing material is required to prevent bacterial infection and preserve moisture.[15–17] It is therefore imperative to develop a durable wound dressing with excellent gas and water permeating/absorbing properties and excellent antibacterial activities.[18,19]

Previous studies showed that heparin could regulate the activities of various proteinases, such as leukocyte elastase,[20–22] trypsin,[23–25] thrombim,[26,27] and so forth. Heparin had been widely used in topical, parenteral application, and in bio-engineered membranes. Recent findings revealed that heparin have many functional properties in uses,[28] such as

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relieved pain, inhibited inflammation and clotting, anticomplementary action,[29] restored blood flow, and improved healing. Heparin, moreover, is an excellent anticoagulant for cell adhesiveness.[30] This study adopted the acrylic acid to graft on the surface of PP nonwoven fabric to undertake a two-step immobilization process. First, the mixtures of collagen/chitosan were employed to immobilize on the surface of AA-grafted PP nonwoven fabric.[14] Second, the immobilizing solutions containing various amounts of heparin were introduced to immobilize on the surface of the collagen/chitosan layer.[30] The parameters on water absorbing and water permeating properties and the antibacterial activity for those composite materials were examined. Meanwhile, the spectra (surface reflectance) of Fourier Transform infrared spectroscopy among polypropylene (PP), AA-grafted polypropylene (PP-AAg), AA-grafted and collagen/chitosan-immobilized polypropylene (PP-AAg-CCi), and AA-grafted and collagen/chitosan-immobilized polypropylene after been immobilized with heparin (PP-AAg-CCHi) were also analyzed for comparison. As a result, this study aims to produce a nonantigenic wound dressing in good resemblance to the dermis of skin with high water absorbing and permeating properties and high antibacterial activities.

MATERIALS AND METHODS

Materials

Polypropylene nonwoven fabric, 50 g/m^2, was supplied by Industrial Technology Research Institute, Taipei, Taiwan. The average denier of the melting blown fiber was about 0.4–0.5 and the fineness of the fiber was 7–8 μm. Glutaraldehyde was employed as a crosslinking agent. Glutaraldehyde and AA were obtained from Acros Organics, Geel, Belgium. Collagen, chitosan, and heparin (Sigma H-6279) were from SIGMA, Louis. Other chemicals used were all reagent grade. The chemical structures of chitosan, collagen, and heparin were listed in Scheme 1.

Methods

Preparation of dressing materials

The initial PP nonwoven fabric samples were pretreated with pure acetone and grafted with AA by the same method as used in the previous study of ours.[14] Then, the AA-grafted nonwoven fabric samples were dried at 105°C for at least 1.5 h to clear the residual water and toluene on the grafted samples, and weighed to obtain the grafting percentage as the following.[14]

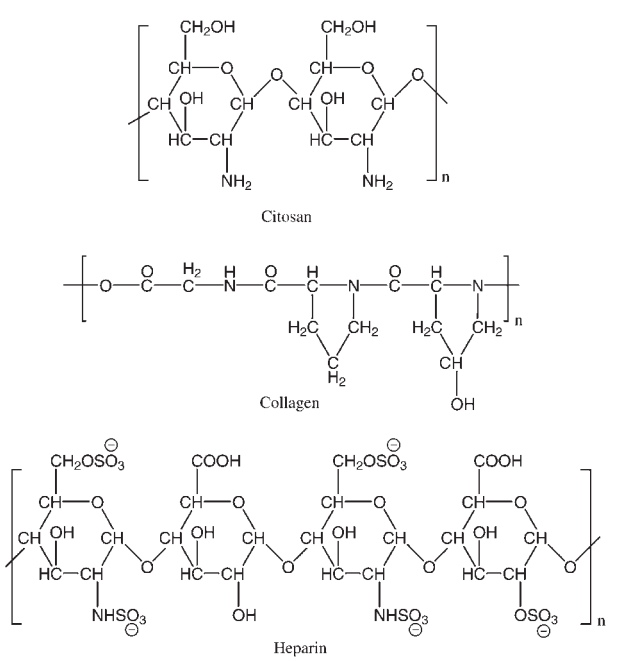
$$\text{Grafting percentage (\%)} = \left(\frac{\text{dry weight after grafting} - \text{dry weight before grafting}}{\text{dry weight before grafting}}\right) \times 100 \quad (1)$$

The grafting percentage of the AA-grafted PP nonwoven fabric for this study was controlled at 15 wt %.

Immobilizing percentage

This study adopted a two-step procedure. In the first step, the grafted fabric samples were immersed in the glass dish containing specific concentrations at 4°C. Then, 18 h to immobilize the various mixed ratios of collagen/chitosan onto those nonwoven fabric samples.[14] Those immobilized fabric samples were next fully washed with cold distilled water, neutralized with 1 wt % sodium hydroxide at room temperature for 30 min, and washed with deionized water in the ultrasonic washing machine for 10 min to remove the unimmobilized agents. Those collagen/chitosan immobilized fabric samples were dried at 35°C in a (40 Torr) vacuum drier for 24 h.

In the second step, the immobilized fabric samples obtained from the first step were introduced into the heparin solutions to proceed the immobilization process. In this step, the collagen/chitosan immobilized fabrics, to be preactivated, were first immersed in



Scheme 1 Chemical structures of chitosan, collagen, and heparin.

the solution of *N*-ethyl-*N*′-(3-dimethyl-aminopropyl) carbodiimide hydrochloride (EDC) at 4°C for 30 min. EDC was dissolved in a buffer solution at pH 4.8. The preactivated fabrics were immersed in the solutions containing various amounts of heparin (0.1, 0.3, 0.5, and 0.7 wt %) at 4°C for 24 h for immobilization. Heparin was dissolved in a buffer solution at pH 7.6. Those samples were then washed with deionized water in the ultrasonic washing machine for 10 min to remove the unimmobilized heparin. Finally, the heparin-immobilized fabric samples were dried at 35°C in a (40 Torr) vacuum drier for 24 h. The immobilizing percentages for both steps were calculated as followed.[14]

$$\text{Immobilizing percentage } (\%) = \left(\frac{\text{dry weight after immobilizing} - \text{dry weight before immobilizing}}{\text{dry weight before immobilizing}}\right) \times 100 \quad (2)$$

Antibacterial activity

Antibacterial property (bacteria inhibition percentage) of the various PP-AAg-CCHi samples was examined by the method of AATCC test method 100-1998. The fabric samples were put into a jar, in which 1.0 mL standard bacterial liquid was contained. The top of the jar was screw tightly to prevent the evaporation of water molecules in liquid to proceed the incubation at 37°C. In this study, *staphylococcus aureus* was employed. The bacteria inhibition percentage was calculated as following equation

$$\frac{(B-A)\times 100}{B}$$

or

$$\frac{(C-A)\times 100}{C}$$

or

$$\frac{[(B+C)/2-A]\times 100}{[(B+C)/2]} \quad (3)$$

A is the number of bacteria for the finished fabric sample after the incubation time period of 18 h, *B* is the number of bacteria for the finished fabric sample after the incubation time period of 0 h, and *C* is the number of bacteria for the nonfinished fabric sample (control) after the incubation time period of 0 h. If the values of *B* and *C* are similar, the larger one will be used. If the values of *B* and *C* are different significantly, the average value of $(B + C)/2$ will be used.

In addition, bacteria inhibition zone was observed and was recorded with photographs. The values of inhibition zone were calculated with the following eq. (4). The calculated value of ''clear zone (*W*)'' is defined as the bacteria inhibition zone.

$$W = \frac{(T-D)}{2} \quad (4)$$

where, *W* is the width of clear zone in mm, *T* is the total diameter of specimen and clear zone, and *D* is the diameter of specimen, respectively.

Measurements of water diffusion coefficient

Water absorbing values were measured using the buffered water at pH 6.8, which was prepared by the method of Sorensen buffer solution (the mixed solution of 5 mL of M/15-Na_2HPO_4 and 5 mL of M/15-KH_2PO_4). The measurement was according with the method described by Kabra et al.[31] For the water absorbing kinetics, the simplified equation of Fick was employed to calculate the water diffusion coefficient as follows.[14,31]

$$M_t/M_\infty = (4/\pi^{1/2})(Dt/L^2)^{1/2} \quad (5)$$

where, *D* is the water diffusion coefficient, *t* is swelling time, and *L* is values of the thickness of the grafted and immobilized nonwoven fabric samples. The values of the water diffusion coefficient were thus obtained from the slope of the linear plots between the values of M_t/M_∞ and the values of $(t/L^2)^{1/2}$ of those fabric samples.

Observation of scanning electron microscope

To confirm and compare the distribution of the immobilizing agents on the PP, PP-AAg, PP-AAg-CCi, and PP-AAg-CCHi samples, the PP nonwoven fabric samples were grafted with 15 wt % of AA, and immobilized first with 70 wt % of collagen/chitosan (33/67), and next with heparin (0.7 wt %). The agent and pore distributions of the dressings were observed with LEO 1530 Field Emission Scanning Electron Microscope (SEM, LEO-1530 Schottky FESEM, Oberkochen, Germany).

Fourier transform infrared spectroscopy

The surface reflectance of infrared spectroscopic measurement of PP, PP-AAg, PP-AAg-CCi, and PP-AAg-CCHi samples were recorded directly on the Fourier transform infrared spectroscopy (FTIR, FTIR Spectrum One Perkin–Elmer, NJ), which is equipped of attenuated total reflectance.

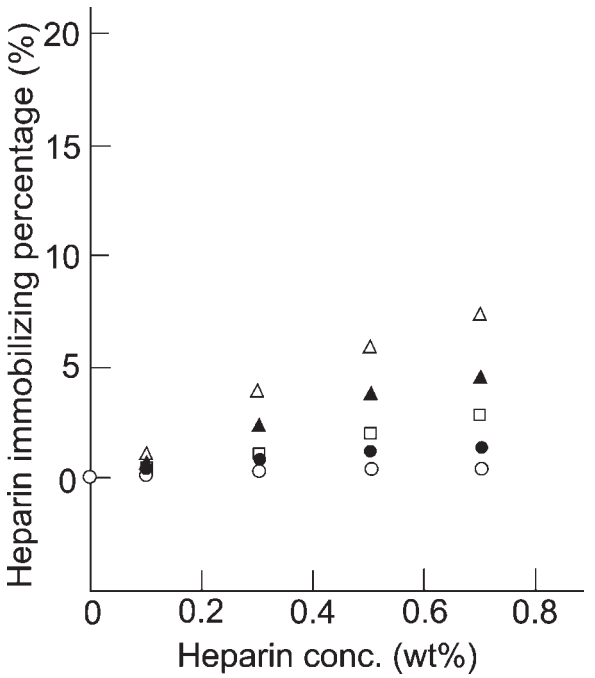


Figure 1 The relationships between the increased immobilizing percentages and the heparin concentrations in immobilizing bath measured from the samples of the various mixed ratios of collagen/chitosan. (△): collagen/chitosan = 0/100; (▲): collagen/chitosan = 33/67; (□): collagen/chitosan = 50/50; (●): collagen/chitosan = 67/33; and (○): collagen/chitosan = 100/0.

RESULTS AND DISCUSSION

Figure 1 representing the values of the heparin immobilizing percentage for the various PP-AAg-CCHi samples (varying the mixed ratios of collagen/chitosan[14]) under different heparin concentrations indicates that the increase of heparin concentration in the immobilizing bath for a given mixed ratio of collagen/chitosan results in the increase of the heparin immobilizing percentages. It is evident that the higher the concentration of heparin, the greater its chance to immobilize on the surface of the PP-AAg-CCi sample. Figure 1 also reveals that the amount of the immobilized heparin becomes larger when chitosan contained in the collagen/chitosan mixture increases. As examined in our previous study,[14] the main difference between collagen and chitosan consists in that the former contains carboxylic acid, amine, and hydroxyl groups, while the latter contains only amine and hydroxyl groups. The containing ratio of the amine group on the chitosan molecule, however, is higher than that of the collagen molecule (Scheme 1). It can thus be inferred that the higher reactivity of the amine group probably results in the larger immobilized amount for the greater chitosan-contained sample.

To ensure the improvement of the water absorbing property of the PP-AAg-CCHi samples, the water absorbing values of the PP-AAg-CCi sample for the mixed ratio of collagen/chitosan at 33/67 were selected and measured for comparison, whose results are shown in Figure 2. After heparin immobilization, the water absorbing property of the PP-AAg-CCi sample turns out to be significantly improved. It also shows that the water absorbing values increase significantly with the heparin concentration contained in the immobilizing bath. This phenomenon is probably caused by the content of the hydrophilic group, $—SO_3$, contained in heparin (Scheme 1). Additionally, Figure 2 shows that the values of water absorbing decrease with the increase of the pH values of the buffered water. This result is precisely in keeping with that in the previous study.[14] The greater water absorbing values, it is accounted, are probably caused by the greater amount of the hydrophilic groups (i.e., cation of ammonium from imine and amine group of chitosan and heparin under the lower pH condition) contained in the immobilized materials (i.e., collagen/

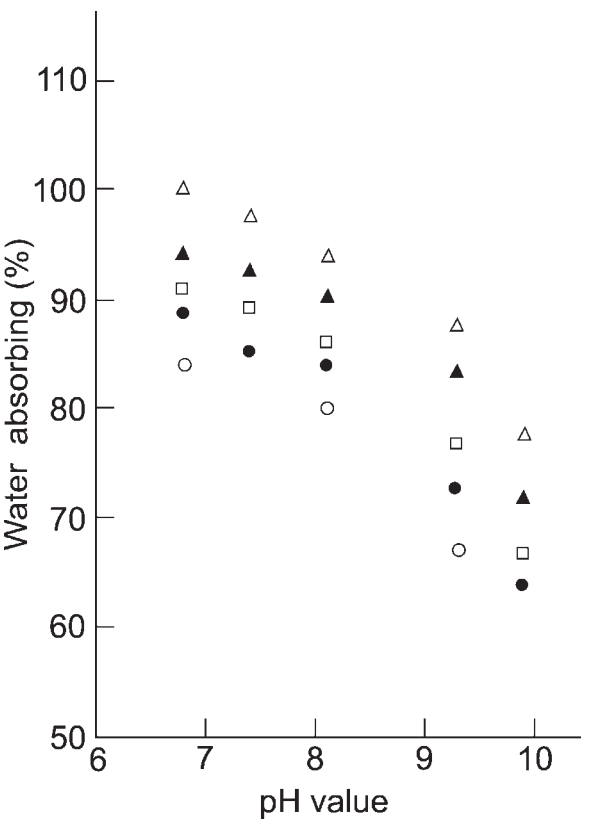


Figure 2 The water absorbing under different pH values of buffered water for the samples of PP-AAg-CCi and PP-AAg-CCHi. The mixed ratio of collagen/chitosan was 33/67. Heparin concentration: (○) = 0 wt %; (●) = 0.1 wt %; (□) = 0.3 wt %; (▲) = 0.5 wt %; (△) = 0.7 wt %. The data for heparin concentration at 0 wt % are obtained from Ref. 14.

chitosan/heparin) under the lower pH medium. Some studies[31,32] pointed out that the higher the pH value of the buffered water, the lower water absorbing property for the crosslinked poly(vinyl alcohol)/chitosan.

The spectra of the surface reflection-infrared spectroscopy (IR) in Figures 3(a–d) are: (a) PP, (b) PP-AAg, (c) PP-AAg-CCi, and (d) PP-AAg-CCHi, respectively. The spectrum of PP in Figure 3(a) reveals two strong absorbing bands at about 1451 and 1372 cm^{-1}, respectively, for the $—CH_2—$ and $—CH_3$ groups of polypropylene.[33] The spectrum of PP-AAg [Fig. 3(b)] reveals the same absorbing band of the $—CH_2—$ and $—CH_3$ groups of polypropylene and the absorbing band of —C—*OH* at about 1117 cm^{-1} and the carbonyl group (—*CO*—) at about 1710 cm^{-1}, simultaneously. That is undoubtedly the carboxylic acid group of acrylic acid grafted on the surface of polypropylene. The spectrum of PP-AAg-CCi [Fig. 3(c)] reveals that the same absorbing bands of the $—CH_2—$ and $—CH_3$ groups of polypropylene still exist and four new absorbing bands at 1640, 1553, 1051, and 1026 cm^{-1} are generated, but the carbonyl group of the acrylic acid at 1710 cm^{-1} weakens slightly and shifts to a lower frequency position. Silverstein et al. description[33] ensures us that the shift from 1710 cm^{-1} to the lower frequency value for the carbonyl group of the grafted polyacrylic acid must be caused by the crosslinking by glutaraldehyde. The new absorbing bands at 1640 and 1553 cm^{-1} are the —CO— and —NH— groups of the amide of collagen and chitosan, respectively. The new absorbing bands at 1051 and 1026 cm^{-1} are the ether group of the pyranose ring of chitosan.[33] It is found that the —C—*OH* at 1117 cm^{-1} almost disappears. These phenomena evidently prove the crosslinking reaction between the glutaraldehyde and collagen/chitosan and the immobilization of collagen/chitosan on the PP-AAg samples. Figure 3(d) reveals a new and important absorbing band at about 1302 cm^{-1}, which we believe, is the sulfone group[33] of heparin. This phenomenon strongly supports the immobilization of heparin on the surface of the PP-AAg-CCi sample.

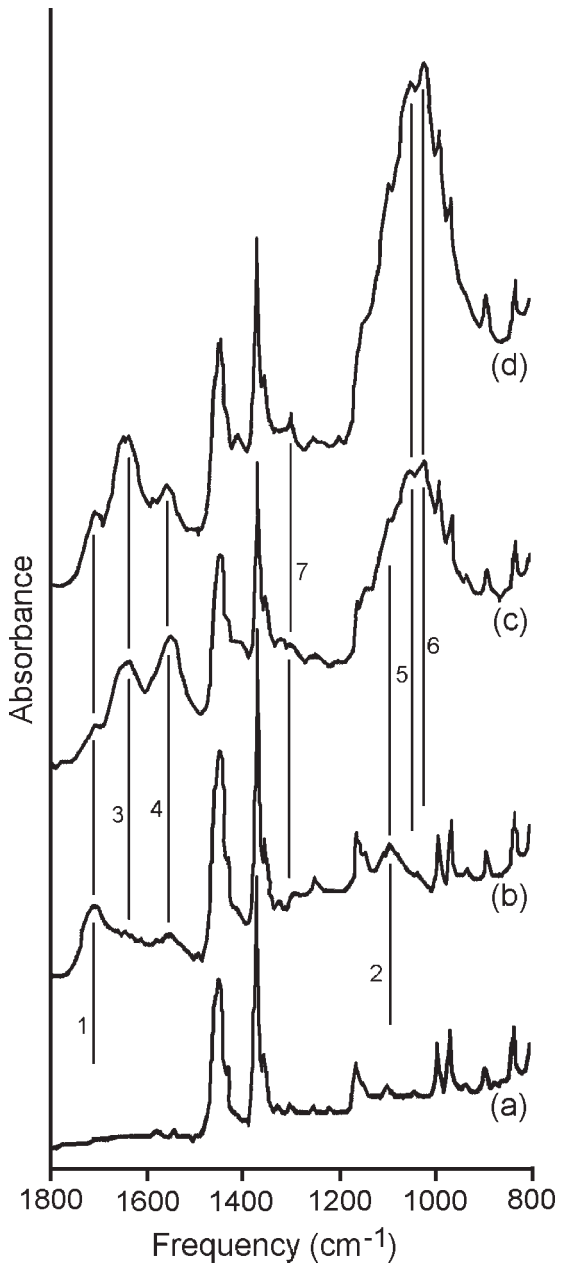


Figure 3 The surface reflection IR spectra of (a) PP, (b) PP-AAg, (c) PP-AAg-CCi, and (d) PP-AAg-CCHi nonwoven fabrics, respectively. Heparin concentration was 0.7 wt %. (1) 1710 cm^{-1}, (2) 1117 cm^{-1}, (3) 1640 cm^{-1}, (4) 1553 cm^{-1}, (5) 1051 cm^{-1}, (6) 1026 cm^{-1}, (7) 1302 cm^{-1}.

The SEM photographs of (a) PP, (b) PP-AAg, (c) PP-AAg-CCi, and (d) PP-AAg-CCHi are shown in Figures 4(a–d), respectively. The pristine PP fiber shows a clear and smooth surface [Fig. 4(a)]; however, after being grafted with AA, the surface of the PP fiber shows sand-like grafting material, which is dispersing and ununiform around the surface of PP fiber [Fig. 4(b)]. As collagen/chtosan is immobilized on the PP sample, there are significant aggregating materials (mass shape) between the PP fibers and on the surface of the PP fiber. The surfaces of those collagen/chitosan mass materials are smooth. The surface of the PP-AAg-CCi sample is changed by heparin immobilization. What interests us is the spot-like materials deposited on the smooth surface of the collagen/chitosan masses. The spot-like materials, it is believed, are the immobilized heparin predicted and expected to change the water-absorbing property (Fig. 2) of the immobilized samples. However, the immobilized heparin does not change the agent and the pore distribution of the PP-AAg-CCi sample.

To further confirm the effect of the immobilized heparin on the surface property of the PP-AAg-

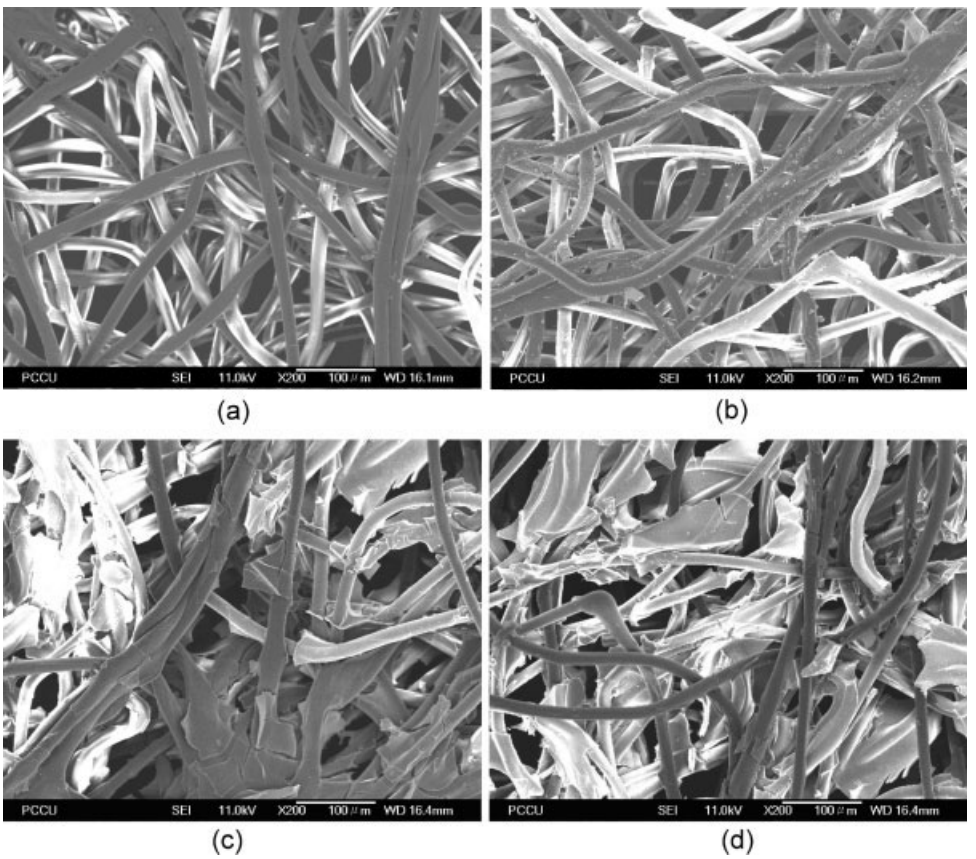


Figure 4 The SEM photographs of (a) PP, (b) PP-AAg, (c) PP-AAg-CCi, and (d) PP-AAg-CCHi nonwoven fabrics, respectively. Heparin concentration was 0.7 wt %.

CCHi sample, the water absorbing kinetics was studied with the buffered water at pH 6.8 employed to be absorbed by the PP-AAg-CCi sample and PP-AAg-CCHi samples. The relationships between the values of M_t/M_∞ and the values of $(t/L^2)^{1/2}$ are indicated in Figure 5, which are plotted according to eq. (5). The linear relationships over the initial absorbing time duration at the conditions of the value of $M_t/M_\infty \leq 0.8$ are found. From those results, we consider it appropriate to use the diffusion kinetic equation described by Kabra and coworkers[31] to study the water diffusion coefficient (D) of the various PP-AAg-CCHi samples. From Table I, which shows the data of the water diffusion coefficient for the various PP-AAg-CCi and PP-AAg-CCHi samples, we can find that the values of the water diffusion coefficient increase significantly with the increasing heparin concentrations in the immobilizing bath. Heparin is a highly hydrophilic material able to increase the value of the water diffusion coefficient.

Table I also lists the values of bacteria inhibition percentage and the bacteria inhibition zone for the various PP-AAg-CCHi samples. From those data, we find that the antibacterial properties (as reflected in the bacteria-inhibition percentages and the bacteria-inhibition zone) are excellent for all the PP-AAg-CCHi samples. The degree of the antibacterial property for the PP-AAg-CCHi sample is the same as that for the PP-AAg-CCi sample. The excellent antibacterial properties of the various PP-AAg-CCHi samples reveal that the immobilized heparin does

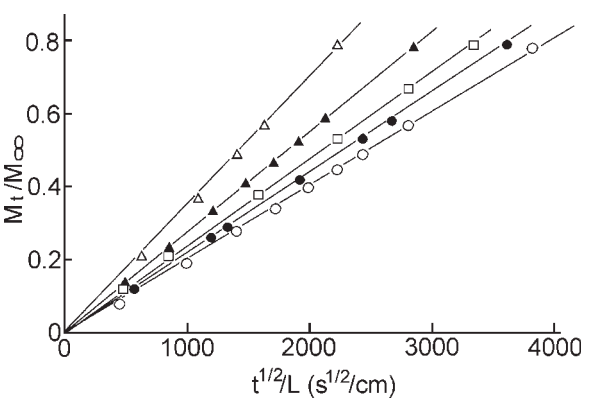


Figure 5 The relationships between the values of M_t/M_∞ and the values of $(t/L^2)^{1/2}$ for the different PP-AAg-CCHi nonwoven fabrics, which were grafted with 15% acrylic acid and 70% collagen/chitosan. The ratio of collagen/chitosan is 33/67. Heparin concentration: (○) = 0 wt %; (●) = 0.1 wt %; (□) = 0.3 wt %; (▲) = 0.5 wt %; (△) = 0.7 wt %. The pH value of buffered water is 6.8.

TABLE I
The Data of Water Absorbing, Water Diffusion Coefficient, Bacteria Inhibition Percentage, and Bacteria Inhibition Zone of AA Grafted and (Chitosan/Collagen)-Heparin Immobilized PP Nonwoven Fabrics Under Different Amounts of Heparin in the Immobilizing Bath

	Properties			
Concentration of heparin (wt %)	Water absorbing (%)	Water diffusion coefficient ($\times 10^8$ cm^2/s)	Bacteria inhibition percentage (%)	Bacteria inhibition zone (mm)
0[a]	83.8	0.89	100.0	12.0
0.1	85.9	0.96	100.0	12.2
0.3	90.5	1.21	100.0	12.1
0.5	94.3	1.51	100.0	12.1
0.7	101.6	1.83	100.0	12.2

[a] The data have been listed in Table I of Ref. 14.

not affect/lower the antibacterial property. Figures 6(a–c) show the photographs of the bacteria inhibition zone for (a) PP, (b) PP-AAg-CCi, and (c) PP-AAg-CCHi, respectively. The zone around the sample is caused by the bacteria inhibition of chitosan.[14] The larger area of zone indicates the higher ability of bacteria inhibition. Figure 6(c) shows unequivocally the higher bacteria inhibition ability of the PP-AAg-CCHi sample, so does Figure 6(b) show the higher ability of the PP-AAg-CCi sample.

From the above, we can conclude that heparin can be immobilized on the surface of the PP-AAg-CCi sample to improve significantly the water absorbing and permeating properties for its higher reactivity and hydrophilic properties. It was discovered that the antibacterial activity of the PP-AAg-CCHi sample is the same as that of the PP-AAg-CCi sample. With many functional properties of heparin applied in the bio-field,[21–30] the products of this multi-layer material of PP-AAg-CCHi (which has been proven successful on producing PP-AAg-CCHi from the heparin immobilization on the surface of PP-AAg-CCi) are expected to render better services for wound dressing.

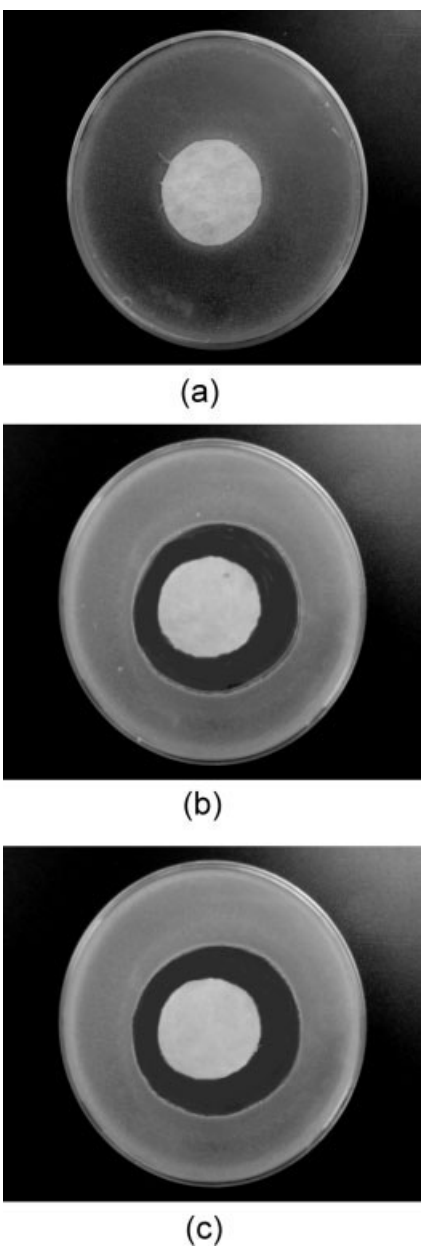


Figure 6 The photographs of the bacteria inhibition zone for (a) PP, (b) PP-AAg-CCi, and (c) PP-AAg-CCHi. Heparin concentration was 0.7 wt %.

CONCLUSIONS

The heparin immobilizing percentages and water absorbing increased with the increasing heparin concentration in the immobilizing bath, while the water absorbing values for the PP-AAg-CCHi samples decreased with the increase of the pH value of the buffered water. The content of chitosan on the collagen/chitosan-immobilized sample could augment the percentages of heparin immobilization, which was confirmed by the absorbing band of the sulfone group of heparin using the surface reflection IR spectra. The values of the water diffusion coefficient also increased with the increasing concentrations of heparin in the immobilizing bath. The bacteria inhibition activities were excellent for both the PP-AAg-CCi and PP-AAg-CCHi samples and reconfirmed as

observed by the photographs of the bacteria-inhibition zone. Likewise, the agent and pore distribution for PP-AAg-CCHi could be observed by the SEM photographs. The improved water absorbing and permeating properties and the excellent antibacterial property of the PP-AAg-CCHi samples has turned out to be precisely as we expected. Wound dressing containing this three-layer structure of PP-AAg-CCHi, it is presumed, will make a promising contribution to the successful application on the production of PP-AAg-CCHi made from the immobilization of heparin on the surface of PP-AAg-CCi.

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