# Preparation and Antibacterial Activity of PET/Chitosan Nanofibrous Mats Using an Electrospinning Technique

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**ABSTRACT:** The nanofiber deposition method, by electrospinning, was employed to introduce antibacterial activity and biocompatibility to the surface of poly (ethylene terephthalate) (PET) textiles. The polymer blends of PET and chitosan were electrospun on to the PET micro-nonwoven mats for biomedical applications. The PET/chitosan nanofibers were evenly deposited on to the surface, and the diameter of the nanofibers was in the range between 500 and 800 nm. The surface of the nanofibers was characterized using SEM, ESCA, AFM, and ATR-FTIR. The wettability of the PET nanofibers was significantly enhanced by the incorporation of chitosan. The antibacterial activity of the samples was evaluated utilizing the colony counting method against *Staphylococcus aureus* and *Klebsiella pneumoniae*. The results indicated that the PET/chitosan nanofiber mats showed a significantly higher growth inhibition rate compared with the PET nanofiber control. In addition, the fibroblast cells adhered better to the PET/chitosan nanofibers than to the PET nanofibers mats, suggesting better tissue compatibility. © 2007 Wiley Periodicals, Inc. J Appl Polym Sci 105: 2816–2823, 2007

**Key words:** nanofiber; polyester; chitosan; antibacterial activity

## INTRODUCTION

Poly (ethylene terephthalate) (PET) is widely used for barriers, fibers, sheets, and films because of its excellent characteristics such as transparency and outstanding processability. It is light in weight, and it has good impact resistance.[1] PET is also used in cardiovascular implants such as artificial heart valve sewing rings and artificial blood vessels.[2] Development of PET textiles, with antibacterial activity, has been tried as an approach for enhancing the functionality and the added value of medical textiles. Antibacterial activity also allows PET textiles to be used in clothing, shoes, bedding, and interior materials for automobiles.[3,4]

Chitosan and chitin are frequently used as antibacterial processing agents. Chitin and chitosan show *in vitro* bacteriostatic, bactericidal, and candidacidal activity. In an *in vivo* study, chitin and chitosan, which were administered intraperitoneally to mice were proved to have a protective influence against infections.[5,6] When chitosan sticks to a bacterial cell wall, it penetrates into the cells and inhibits the growth of cells by preventing the transformation of DNA into RNA. As a result, it can suppress the metabolism of bacteria.[7] Chitosan, however, is disadvantageous for a wide array of applications because it swells readily in water, it can be easily removed from the surface of matrix materials and is not easily solution processed. Blending chitosan with PET might enhance antibacterial activities and biocompatibility to PET leading to novel materials for applications of textiles. Moreover, adding PET could make up for the poor processability of chitosan by its swelling in water.

Nanofibers are ultrafine solid fibers notable for their very small diameters. They have significantly large surface area to volume ratio, porosity, surface functionality, and superior mechanical properties.[8,9] These remarkable properties underlie the substantial interest in nanofibers for industrial, biomedical, and electronic applications.[10,11] Electrospinning has been considered as one of the most useful ways to fabricate nanofibers due to its simple procedure. The polymer filaments are formed between two electrodes, the solution and a collector, which bear electrical charges of opposite polarity. Once ejected out of a syringe through a needle, the charged solution jets evaporate to become fibers that can be deposited on to the collector.[12–14]

Recently, the fabrication of both PET and chitosan nanofibers has been attempted for their biomedical

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potential. The PET nanofibers have the potential to be used as blood vessels[15] while chitosan nanofibers can be used as an extracellular matrix (ECM) and in drug dosage forms.[16,17] One of the methods that introduce antibacterial activity to the PET textile can generate functional groups on to the surface of PETs by using gas plasma or formaldehyde treatment. This is subsequent to grafting antibacterial agents on to the activated surface of PETs.[3,4,18] However, the referred method has the complex procedures such as several steps of chemical treatments.

In this study, nanofiber deposition by electrospinning was employed to introduce antibacterial activity to the surface of PET textiles. Here, we report on the preparation of the PET nanofibrous scaffolds that contain chitosan on the PET textiles through an electrospinning technique. The PET/chitosan nanofibrous scaffolds were characterized by atomic force microscopy (AFM), electron spectroscopy for chemical analysis (ESCA), and a water contact angle goniometer. The effect of the PET/chitosan matrices on the bacteria growth inhibition rate and cell adhesion was also examined.

## EXPERIMENTAL

### Materials

The PET chips and micro-nonwoven mats used in this study were supplied by Saehan (Kumi, Korea). Chitin powder, hexafluoropropanol, and trifluoroacetic acid were purchased from the TCI (Japan). Chitosan (85% deacetylated, viscosity (Brookfield, 1% solution in 1% acetic acid) >20 cPs) was purchased from Aldrich. The mouse fibroblasts and the bacteria, *Staphylococcus aureus* and *Klebsiella pneumoniae*, were obtained from the Korean cell line bank (KCLB) and the Korean collection for type cultures (KCTC), respectively.

### Purification of the chitosan

Chitosan was dissolved in a 2% acetic acid aqueous solution for 24 h and filtrated thru a glass frit to remove the insoluble part. The chitosan solution was then precipitated in a cold 1*M* NaOH aqueous solution. The precipitated chitosan was washed with distilled water and then dried under a vacuum for 24 h.

### Electrospinning of nanofiber mats

For electrospinning, the PET/chitosan and PET/chitin were dissolved in trifluoroacetic acid and hexafluoropropanol, respectively. The electrospinning apparatus used in this study consists of a glass syringe attached with a capillary tip (18G), a syringe pump, a collector, and a high-voltage supply. The needle tip is connected to a high-voltage supply and the polymer solution was charged and ejected. The nanofibers were deposited on to the PET micro nonwoven mat, which was adhered to the collector. The polymer solutions were electrospun with a mass flow rate between 0.3 and 0.7 mL/h and a voltage between 12 and 20 kV. The distance between the tip and the collector was in the range of 10–15 cm. The electrospun samples were dried under a vacuum 20°C for 24 h.

### Surface characterization method

The morphology of electrospun nanofibers was observed using a field-emission scanning electron microscope (FE-SEM, Hitachi *S*-4300, Japan) after gold coating. The samples were analyzed using ESCA (ESCA LAB VG microtech, Mt 500/1 etc, East Grin, UK) equipped with Mg Kα at 1253.6 eV and 150 W power at the anode and the surface elemental compositions relative to carbon were calculated from the peak height with a correction for atomic sensitivity. Surface properties of the nanofibers were examined using a Nanoscope IIIa atomic-force microscope (Digital Instruments, Santa Barbara, CA), in phase mode, in air, and using a square pyramidal tip of a $Si_3N_4$ cantilever. In phase mode imaging, the phase shift of the oscillating cantilever shows specific material properties effecting interaction between the tip and samples.

The wettability of fibers was investigated by the visual observation of the water drop on the surface of nanofiber mats.

### Antibacterial assessment

*Staphylococcus aureus* and *Klebsiella pneumoniae* were cultivated in a nutrient broth for 24 h in a $CO_2$ incubator. The nanofiber mats were sterilized in an autoclave and cut to sizes of $1 \times 1$ cm$^2$. The diluted bacteria suspension was cultured in a vial containing 0.4 g of the samples. The vials were incubated at 37°C for 18 h. The bacteria collected from each vial were plated on to the agar medium. After incubation at 37°C for 24 h, the resulting bacterial colonies in the plates were counted visually. The percentage of the bacterial growth inhibition was calculated using the difference between the numbers of colonies from bacteria with samples and those from bacteria in the blank vials.

### Cell adhesion

The NIH 3T3 fibroblasts were cultured in a DMEM supplemented with a 10% FBS and a 1% PGS at 37°C in a $CO_2$ incubator. The cells were collected after treatment with trypsin-EDTA. One milliliter of each cell suspension ($3 \times 10^5$ cell mL$^{-1}$) was added to the nanofiber mats, which were then placed on to a 24-well plate and kept in a $CO_2$ incubator at 37°C. After the 3 h incubation period, the supernatant was removed, washed with a PBS, and fixed by dipping the nano-

fiber mats in a 2.5% glutaldehyde aqueous solution for 1 h. The sample was then dehydrated in a graded series of ethanol, dried in a Hitachi model HCP-2 Critical Point Drier using liquid $CO_2$ as the transition liquid, and finally sputter-coated with gold. The samples were then observed with a Hitachi *S*-510 scanning electron microscope. During the procedure of antibacterial assessment and cell culture, chitin, and chitosan nanofibers were immersed in aqueous medium for several hours. However, they easily lost their mechanical property and were partially dissolved in the aqueous solution because of the swelling property of chitin and chitosan molecules. Using chitin and chitosan nanofibers blended with PET, we observed that chitin and chitosan in blended fibers were not swelled and kept their fiber structure during soaking them in medium.

### MTT test

A 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) (Sigma, MO) assay was used to measure relative cell viability. After a 3 day incubation period for nanofibrous scaffolds, cell viability was evaluated using MTT assay. Fifty microliters of a MTT solution (5 mg/mL in PBS) were added to each well and incubated in a humidified atmosphere of 5% $CO_2$ at 37°C for 4 h. After removing the medium, the converted dye was dissolved in acidic isopropanol (0.04*N* HCl-isopropanol) and kept for 30 min in the dark, at room temperature. From each sample, the medium (100 μL) was taken and transferred to a 96-well plate and subjected to ultraviolet measurements for converted dye, at a wavelength of 570 nm, on a kinetic microplate reader (EL × 800, Bio-T® Instruments, Highland Park).

## RESULTS AND DISCUSSION

### The electrospining of PET and chitosan

The nanofibrous morphology of poly(ethylene terephthalate) and/or chitosan was observed using a scanning electron microscope (SEM). The polymer solutions were individually electrospun under various parameters of polymer concentration and voltage to obtain the maximum conditions for the fabrication of nanofiber mats. For the results, the most suitable nanofiber mat was obtained at a concentration of 15 wt % and a voltage of 15 kV. Figure 1 shows the SEM images of nanofiber mats that are obtained from a hexafluoropropanol solution. The images showed continuous fiber morphology, and the fine fibers were intersected with thick fibers. This was probably due to the different solubility and the dielectric constant of the PET and chitin. Kim et al. fabricated electrospun poly (ethylene terephthalate) and poly (ethylene naphthalate) and examined their thermal properties.[19] They reported that the electrospinning of polymers resulted in an increase of crystallinity and a decrease in the glass transition. Figure 2 shows the SEM images of the nanofiber mats that are obtained from trifluoroacetic acid solution. Spasova et al.[17] and Park et al.[20]

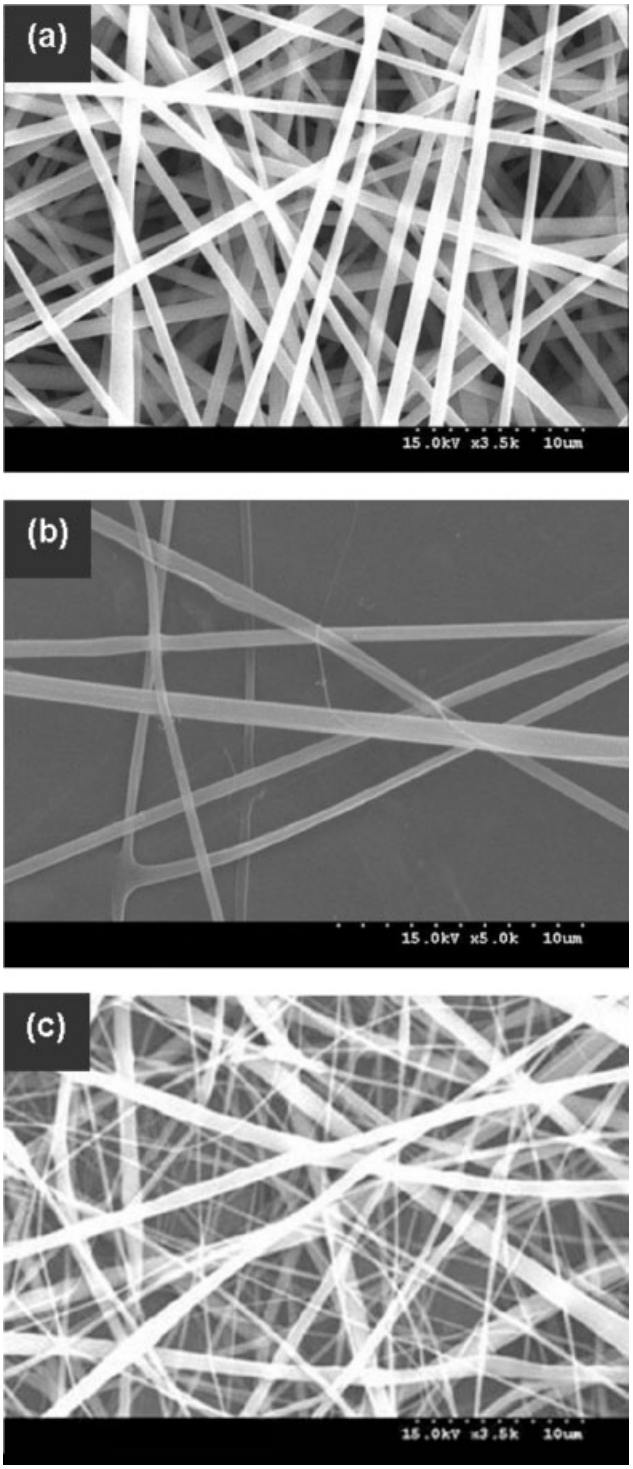


**Figure 1** SEM images showing electrospun nanofibers obtained from hexafluoropropanol solutions: (a) PET, (b) chitin, (c) PET/chitin.

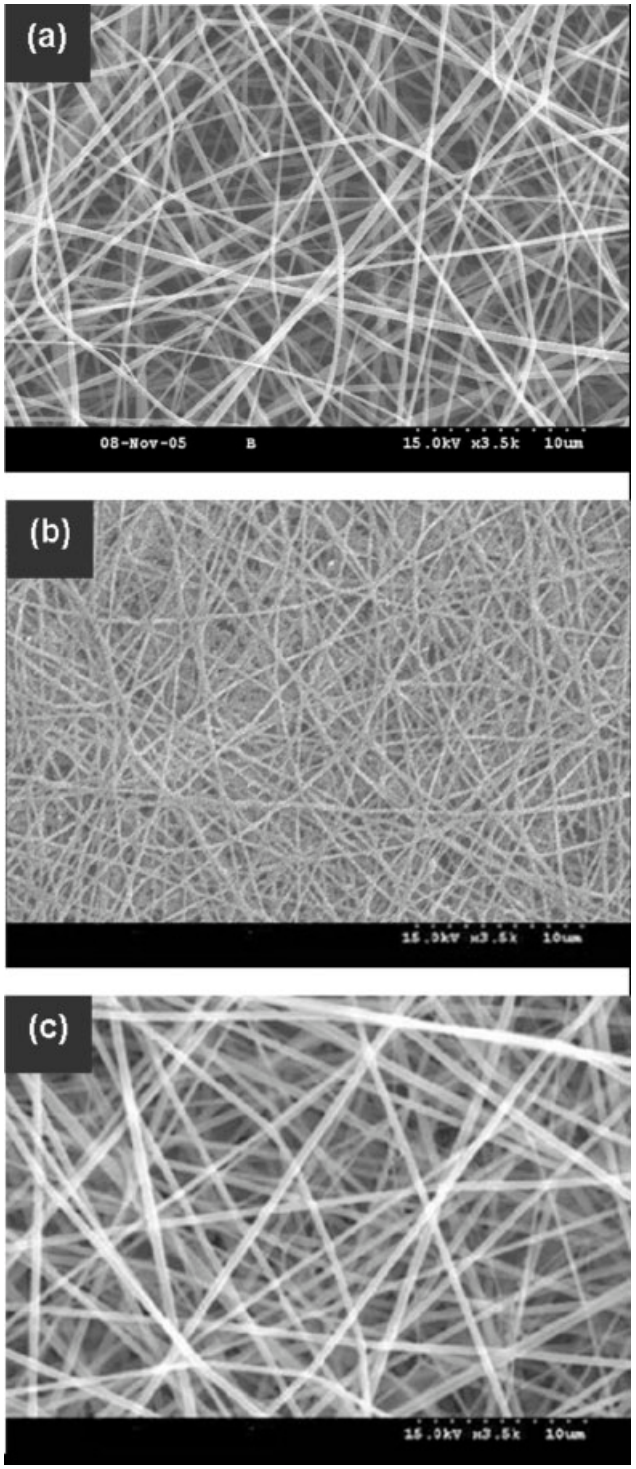


**Figure 2** SEM images showing electrospun nanofibers obtained from trifluoroacetic acid solutions: (a) PET, (b) chitosan, (c) PET/chitosan.

prepared chitosan nanofibers that were blended with PEO and silk fibroin, respectively. Geng et al.[21] prepared electrospinning of chitosan solutions in acetic acid and reported that the average diameters were distributed from 40 to 130 nm, depending on the concentration of acetic acid. We prepared chitosan nanofibers from trifluoroacetic acid solution. As a result, the diameter of PET [Fig. 2(a), 300–500 nm] fibers obtained from 15 wt % trifluoroacetic acid solution was smaller than those [Fig. 1(a), 500–1200 nm] obtained from 15 wt % hexafluoropropanol solution and this came from the viscosity difference of two solutions. The viscosity of hexafluoropropanol solution (11.5 cPs) was higher than that of trifluoroacetic acid solution (5.2 cPs) and the viscosity of solutions can strongly affect the morphology of electrospun nanofibers. When compared with the fiber image of PET/chitin [Fig. 1(c)], PET/chitosan showed a narrow diameter distribution, as represented in Figure 2(c).

### Characteristics of nanofibers

To examine the surface morphology of the nanofibers, an atomic force microscope (AFM) image was studied using a tapping mode and it was expressed in the form of a phase image, as shown in Figure 3. In phase mode imaging, the phase shift of the oscillating cantilever relative to the driving signal is measured. If two

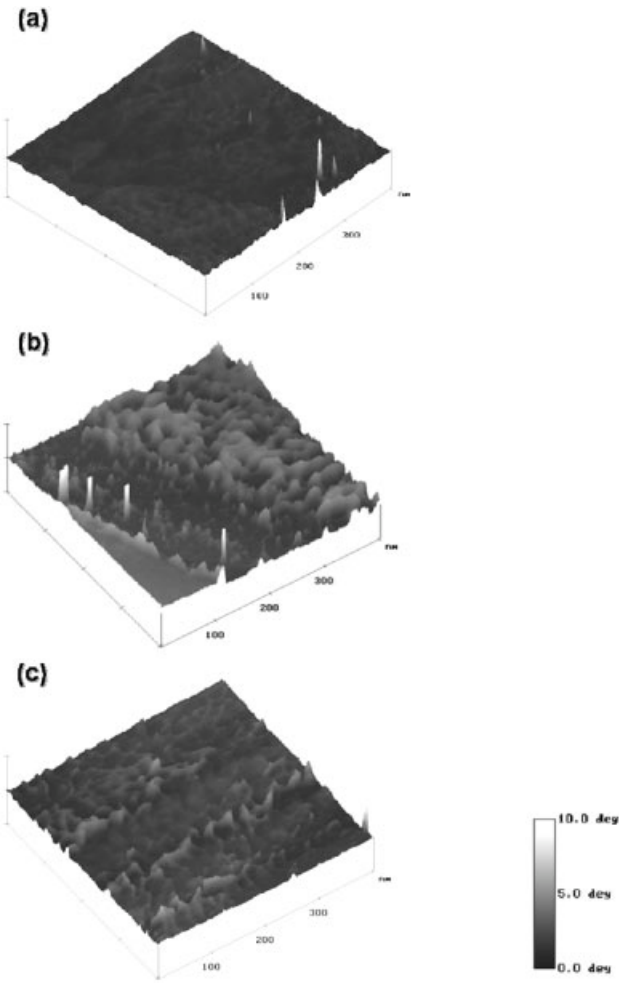


**Figure 3** AFM images of the nanofibers obtained by phase mode: (a) PET, (b) PET/chitin, (c) PET/chitosan.

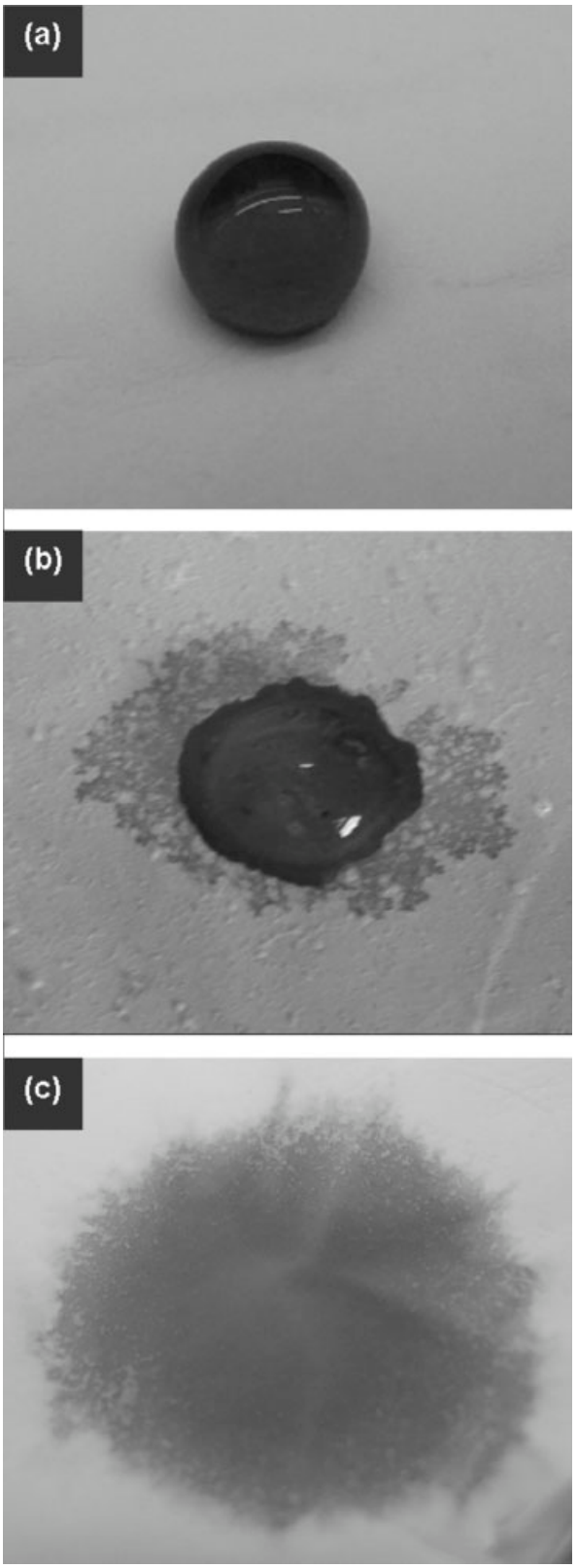


**Figure 4** Water drop containing phenol red on the PET (a), PET/chitin (b), and PET/chitosan nanofiber (c) mats after 5 s.

chemically different molecules exist on the surface, the interaction between a tapping cantilever and materials become different and the separated image appears. Therefore, the separated color of the phase mode indicates the presence of different chemical substances on the surface of materials. For the results, the PET nanofiber surface (a) showed a relatively homogeneous color pattern while PET/chitin (b) and PET/chitosan (c) showed heterogeneous color patterns due to their different chemical composition, thus showing chitin and chitosan were well distributed in the PET fibers. Surface wettability was evaluated by placing a stained water drop on the nanofiber mat and observing the drop visually (Fig. 4). The water drop on the PET nanofiber mat maintained a spherical shape for a long time. However, a waterdrop on the PET/chitosan nanofiber mats spreaded immediately while waterdrop on PET/chitin did slowly. Blending chitosan improved hydrophile property to PET surface much more than blending chitin. This result suggests that the PET nanofiber mat became more hydrophilic after the incorporation of chitosan into the PET fibers.

Changes in the chemical structure of the nanofibrous mats were investigated using ESCA. The PET nanofiber mat containing 10% of chitosan showed three peaks corresponding to C1s (binding energy, 285 eV), N1s (binding energy, 400 eV), and O1s (binding energy, 532 eV), while the PET nanofiber mat showed two peaks corresponding to C1s and O1s. The chemical composition of the nanofibrous scaffolds, calculated from the ESCA survey scan spectra, are shown in Table I. The oxygen content (41.2%) of the PET nanofiber surface decreased to 37.4% due to the incorporation of chitosan (PET/chitosan), while nitrogen was newly observed at the level of 0.9%, indicating the presence of chitosan on the surface. The result also showed that 10% chitin was well blended with PET nanofibers. Nitrogen was found in PET/chitin nanofibers (1.0%) due to 4.0% of nitrogen contained in chitin while not found in PET. However, the nitrogen content of PET/chitosan (0.9%) and PET/chitin (1.0%) shown in Table I was larger than those calculated (PET/chitosan: 0.42%, PET/chitin: 0.43%). The result

**TABLE I**
**Elemental Composition of the Nanofibers Calculated from ESCA Survey Scan Spectra**

| | Atomic percent (%) | | |
|---|---|---|---|
| Substrates | C | O | N |
| PET | 58.8 | 41.2 | – |
| PET/chitosan | 61.7 | 37.4 | 0.9 |
| Chitosan | 46.1 | 49.7 | 4.2 |
| PET/chitin | 61.7 | 37.3 | 1.0 |
| Chitin | 46.2 | 49.8 | 4.0 |

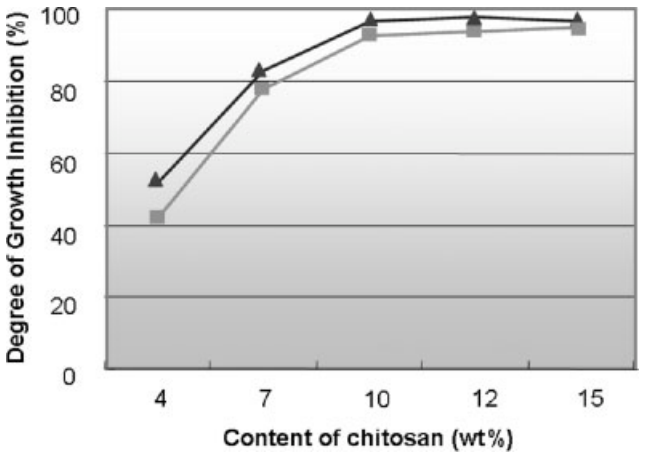


**Figure 5** The effect of chitosan content on bacteria growth inhibition of PET/chitosan nanofiber mats: ▲; *Staphylococcus aureus*, ■; *Klebsiella pneumoniae*.

indicated that chitosan and chitin were more distributed on the fiber surface than the bulk.

### The antibacterial activity of nanofiber mats

The antibacterial activity of nanofiber mats was evaluated by counting the colonies that formed on the plates, and the degree of growth inhibition was calculated from the following equation.

$$\text{Degree of Growth Inhibition} = \frac{B - A}{B} \times 100$$

where $A$ and $B$ are the number of colonies from the bacteria in the vial with samples and the vacant vial, respectively. Fu et al. prepared poly (ethylene terephthalate) film with multilayer of chitosan and heparin, and reported that it exhibited good antiadhesion and antibacterial property against *Escherichia coli*.[22] Yang et al. prepared chitosan-modified nonwoven PET, which showed limited biocidal effect.[4]

The degree of bacteria growth inhibition of *Staphylococcus aureus* and *Klebsiella pneumoniae* was carried

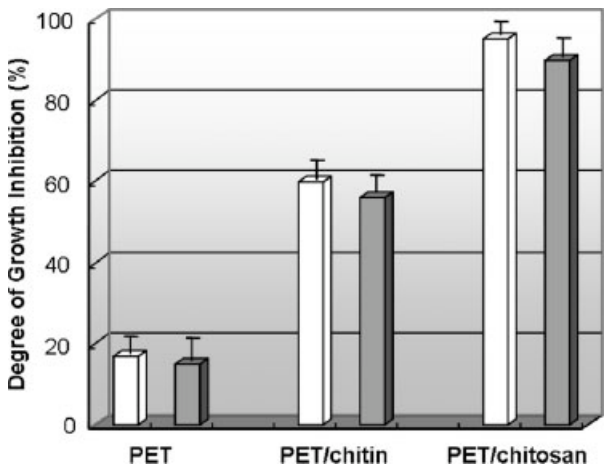


**Figure 6** Degree of growth inhibition of PET, PET/chitin, and PET/chitosan nanofiber mats against *Staphylococcus aureus* (□) and *Klebsiella pneumoniae* (■).

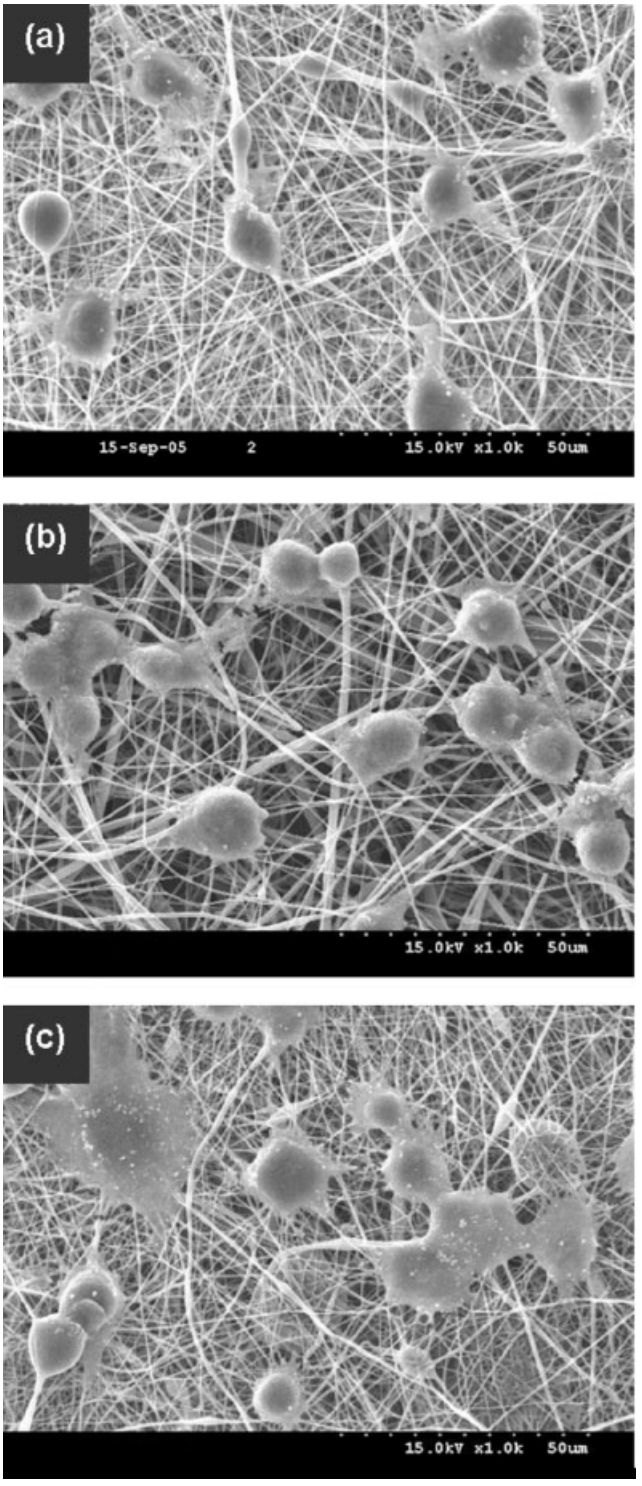


**Figure 7** SEM images of NIH3T3 fibroblasts seeded on nanofiber mats after 3h culture : (a) PET, (b) PET/chitin, (c) PET/chitosan.

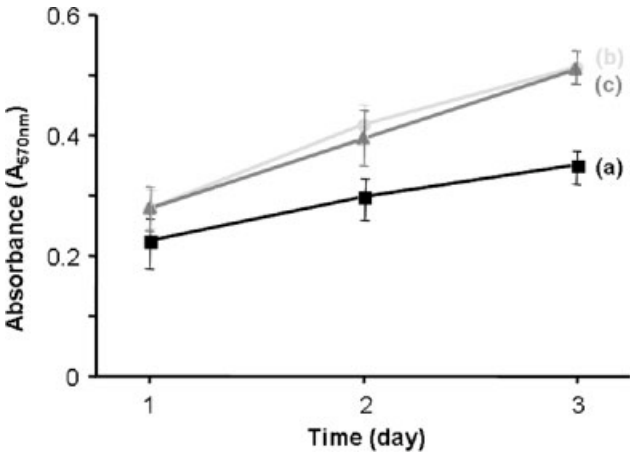


**Figure 8** MTT reduction obtained from the NIH3T3 fibroblasts cultured on the nanofiber mats as a function of incubation time: (a) PET, (b) PET/chitin, (c) PET/chitosan.

out by chitosan content of PET/chitosan nanofiber mats and the result was shown in Figure 5. The growth inhibition of bacteria gradually increased with an increase in chitosan content and it reached a maximum value when the chitosan content is 10 wt %. From the result, 10 wt % of chitosan was chosen to blend with PET to be fabricated as PET/chitosan nanofibers and the content of chitin used as control was 10% as well.

Figure 6 shows the growth inhibition of the PET, PET/chitin, and PET/chitosan nanofiber mats against *S. aureus* and *K. pneumoniae*, respectively. The electrospun PET, which was blended with 10 wt % chitosan, exhibited a degree growth inhibition of more than 95% while PET alone showed a degree growth inhibition under 20%. The degree growth inhibition of 10 wt % of chitin blended with PET was around 60%, not as high as that of chitosan. Adding chitosan to PET textiles showed significant improvement of antibacterial activity, much higher than adding chitin, on both bacteria and gave high functionality of textiles.

### Cell behaviors on the nanofibers

Figure 7 shows the SEM images of the NIH 3T3 fibroblasts that adhered to the nanofibrous scaffolds when cultured in a Dulbecco's modified eagle medium containing 10% fetal bovine serum for 3 h. The cells were well adhered to the surfaces of the PET, PET/chitin and PET/chitosan nanofiber mats because of its similar structure to ECM. On the surface of nanofibers containing chitosan and chitin, not only were more cells attached but also the cells were more spread than those on the PET nanofibers, showing high biocompatibility. Chitosan has been used as a surface modifier to improve the cytocompatibility of poly(L-lactic acid).[23] Ma et al. prepared nonwoven polyethylene terephthalate nanofiber mats by electrospinning technology and they were surface modified with gelatin to mimic the fibrous proteins.[15] They reported that the gelatin grafting method can improve the spreading and proliferation of the endothelial cells on to the PET nanofiber mats. Bhattarai et al. prepared chitosan nanofibers, which promoted the attachment of human osteoblasts and chondrocytes and maintained cell morphology and viability of cells.[16] Figure 8 shows the cell viability of fibroblasts that had been cultured for three days on nanofibrous mats. The cell viability on the PET/chitosan was almost the same as that on PET/chitin, but it was higher than that of the PET control.

## CONCLUSIONS

Both PET and chitosan were dissolved in trifluoroacetic acid and the polymer blend solution was electrospun on to the surface of the microsized PET textiles to produce composite nanofibrous mats. The PET/chitosan nanofibers were evenly deposited on to the surface and the diameter of the fibers was in the range between 500 and 800 nm. It was found, from the water spreading experiment, that the PET nanofiber mat became very hydrophilic when it was blended with chitosan. In the experiment regarding antibacterial activity using *Staphylococcus aureus* and *Klebsiella pneumoniae*, the PET/chitosan nanofiber mats showed a significantly high degree of growth inhibition compared with the PET and PET/chitin nanofibers, suggesting that it has a high-potential to be used as antibacterial material.

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