

Two-Nozzle Electrospinning Fabrication of Bicomponent and Bimodal Nanofibrous Mats with Antibacterial Properties

*Leonard D. Tijjing^{1),#)} Michael Tom G. Ruelo¹⁾, Altangerel Amarjargal²⁾, Hem Raj Pant²⁾, Chan-Hee Park²⁾, Zhe Jiang²⁾, Seung-Ji Kang²⁾, Eun-Kyu Kyo²⁾, Bo-sang Hwang¹⁾, Tae-Hyung Kim¹⁾, Dong Hwan Lee¹⁾, Cheol Sang Kim^{1),2) ##)},

¹⁾ *Division of Mechanical Design Engineering* ²⁾ *Department of Bionanosystem Engineering, Chonbuk National University, Jeonju, Jeonbuk 561-756, Korea*
^{#)} ltijing@jbnu.ac.kr, ^{##)} chskim@jbnu.ac.kr

ABSTRACT

The one-step electrospinning fabrication of novel materials with added functionalities is being widely studied because of their wide array of applications. In this paper, we demonstrate a facile way to fabricate hybrid nanofibrous mats with antibacterial functionality by two-nozzle electrospinning. Using PEO both as reducing agent of AgNO₃ and template for Ag NPs, we could utilize the two-nozzle electrospinning to selectively functionalize bicomponent nanofibers (i.e., made of PU and PEO) for making new materials. Here, the larger PU nanofibers give structural stability and prolonged use of the hybrid materials, while the thinner PEO nanofibers provide more reactive surface area, and the in situ decoration of Ag NPs on PEO surfaces gives strong antibacterial functionality to the hybrid mat. The present technique of making hybrid mat has potential for water treatment applications.

1. INTRODUCTION

Among the current studies, the electrospinning fabrication of hybrid materials by blending two or more polymers or the incorporation of metal nanoparticles (NPs) in/on polymer nanofibers have gained vast attention (Zhang et al. 2005; Nataraj et al. 2009; Nataraj et al. 2009; Deniz et al. 2011). Recently, multi-spinneret electrospinning presents itself as an excellent method to fabricate hybrid materials with improved properties by utilizing the different properties of each component polymer (Kidoaki et al. 2005; Sun et al. 2010). In this study, we present a one-step fabrication of hybrid nanofiber mat composed of two different polymeric nanofibers (PEO and PU), wherein only PEO is decorated with Ag NPs without using any chemicals, i.e. PEO is used as both the reducing agent of silver nitrate (AgNO₃) and the template of the formed Ag NPs. Here, the combination of PU and Ag/PEO nanofibers to make into a bicomponent and bimodal hybrid mat is potentially useful as antibacterial tissue scaffolds or for wound-healing applications. The bigger PU nanofibers give structural stability and

prolonged use of the hybrid materials (Hu et al. 2011), while the presence of thinner PEO nanofibers provide more reactive surface area, and the decoration of Ag NPs on PEO surfaces without the use of chemicals gives antibacterial functionality to the hybrid mat.

2. MATERIALS AND METHOD

We prepared two separate solutions two-nozzle electrospinning. In Solution A, 3.3 wt% poly(ethylene oxide) (PEO, molecular weight (MW) = 60 kDa, Acros Organics) and 5 wt% silver nitrate (AgNO_3 , 99.8%, Showa) relative to the weight of PEO, were dissolved in distilled water (DW) in two separate glass containers. In one container, 2 g PEO was dissolved in 48 g DW, and in another container, 100 mg AgNO_3 was dissolved in 10 g DW. After complete dissolution, the two solutions were combined by magnetic stirring in covered containers and kept away from light for the reduction of AgNO_3 into Ag NPs. The ratio of AgNO_3 to PEO in the mixed solution was 1:20. In Solution B, 10 wt% of polyurethane (PU, Skythane[®] X595A-11, Lubrizol) was dissolved overnight in solvent mixture of N,N dimethylformamide (DMF, Showa) and methyl ethyl ketone/2-butanone (MEK, extra pure, Junsei) (50/50, wt: wt%).

Electrospinning was carried out using a multinozzle electrospinning system with robot-controlled, movable and tunable spinnerets made by our laboratory (Figure 1). Two solutions were each collected separately in 10 ml plastic syringe connected to two spinneret holders with 21G needle tip (inner diameter = 510 nm). The two nozzles were oriented horizontally on one side of the collector at an angle of 80° between them. The two syringes were fixed in a syringe pump set at a constant feed rate of 1.0 mlh^{-1} . Each needle tip was connected to two separate power supplies with identical voltages of 15 kV. The grounded collector was made of aluminium plate covered with Teflon sheet, and the tip-to-collector distance was kept at 20 cm for all tests. For comparison, neat PU nanofiber was also prepared by conventional single nozzle electrospinning, using the same conditions above. After electrospinning, the nanofibrous membrane was dried in a dry oven at 60°C for 48 h.

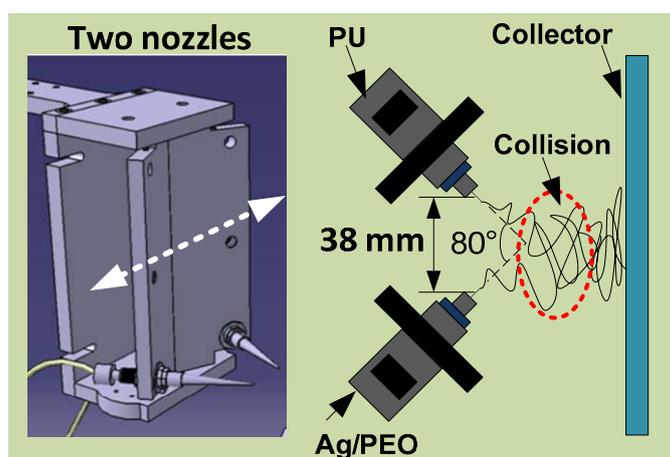


Fig. 1. The present schematic of two-nozzle electrospinning.

The morphological structure of the samples was characterized by field emission scanning electron microscopy (FESEM, Hitachi S-4800), and transmission electron microscopy (TEM, H-7650, Hitachi). Elemental analysis was carried out by energy dispersive X-ray spectroscopy (EDS). Fourier transform infrared (FTIR) spectra of the samples were obtained using a Paragon 1000 Spectrometer (Perkin Elmer). The ultraviolet (UV)-visible spectra of the samples were obtained with a Lambda 900 UV-vis spectrometer (Perkin-Elmer) over the range of 200 – 800 nm.

The antibacterial activity of composite nanofibers against *Escherichia coli*, a Gram-negative bacteria, was investigated by a zone inhibition method. The nanofiber mat samples were formed into circular discs with a diameter of 6 mm. Using a spread plate method, nutrient agar plates were inoculated with 1 ml of bacterial suspension containing around 10^5 colony forming units for each bacteria. The same amounts of samples were gently placed on the inoculated plates, and were then incubated at 37°C for 24 h. Zones of inhibition were determined by measuring the clear area formed around each sample.

3. RESULTS AND DISCUSSION

Electrospinning of hybrid mats was carried out at two different reduction times of AgNO_3 in PEO: 3 h and 5.5 h (referred herein as Hybrid 1 and Hybrid 2, respectively). A study by Saquing et al (Saquing et al. 2009) showed that high-molecular weight PEO has the capability of reducing AgNO_3 into Ag NPs at ambient conditions. The reduction of AgNO_3 into Ag NPs in high MW PEO is attributed to the formation of pseudo-crown ethers that can bind silver ions within the coiled polyether helix, and these bound silver ions are reduced via oxidation of PEO fragments by the metal center (Longenberger and Mills 1995; Saquing et al. 2009). The reason for the conformation similar to that of the crown ethers is the ion-dipole interaction between the metal ion and the electron pair of the ether oxygen in the PEO fragments (Voronov et al. 2008). Furthermore, high MW PEO has longer polymer chains, which in turn can form more crown ether cavities and provide more reaction sites thus making it faster in reduction rate, comparing to using low MW PEO, which require at least 20 days of reduction time as reported elsewhere (Longenberger and Mills 1995). The electrospun nanofibrous mats (Figure 2a – c) all showed highly-porous structure, and randomly-oriented, ultrafine nanofibers. The neat PU nanofibers showed continuous and smooth fibrous structure with an average fiber diameter of 950 ± 270 nm. On the other hand, the hybrid Ag/PEO – PU nanofibers showed two distinct fiber sizes, i.e., larger fiber sizes for PU nanofibers and much smaller sizes for PEO nanofibers. In Hybrid 1 (reduction time of 3 h, Figure 2b), the PU and PEO nanofibers showed an average diameter of 800 ± 290 nm and 194 ± 40 nm, respectively. A closer look at the PEO nanofiber (inset of Figure 2b) shows very small, well-dispersed Ag NPs with diameters ranging from 6 - 10 nm. Similar structures in Hybrid 1 were observed in Hybrid 2 (Figure 2c) but the Ag NPs were larger in diameter ($d = 90 \pm 30$ nm) and had wider diameter distribution. These large Ag NP sizes are attributed to the aggregation of metallic silvers to form clusters, due to their high surface energy (Shi et al. 2011). Thus, for longer reduction time, there is more chance of clusters to aggregate more and form into bigger particles as observed in the present study.

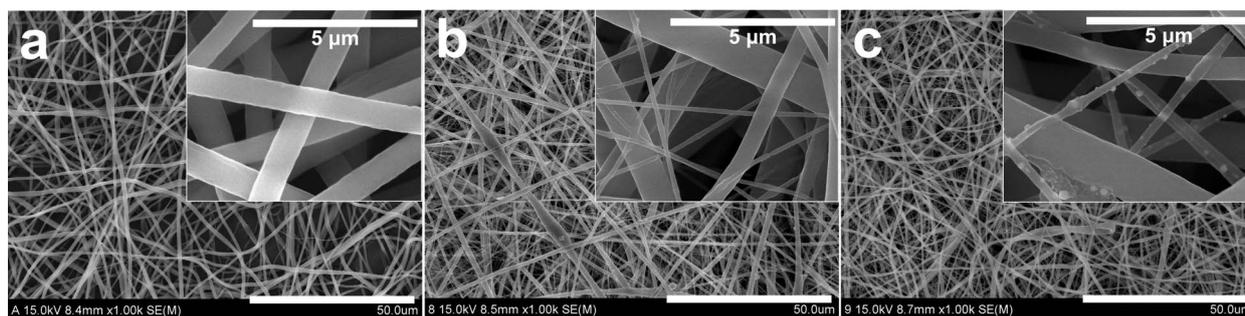


Fig. 2. Low and high (insets) resolution SEM images of: (a) neat PU mat; (b) PU-Ag/PEO (3 h), and; (c) PU-Ag/PEO (5.5 h) composite mats.

To analyze the composition of electrospun nanofibers, we took FTIR spectra of the nanofibrous mats (Figure 3). The characteristic peaks of neat PU nanofibers can be assigned as: 2955 cm^{-1} (CH_2 asymmetric vibration); 2881 cm^{-1} (CH_3 symmetric vibrations); 1733 cm^{-1} (free $\text{C}=\text{O}$); 1709 cm^{-1} ($\text{C}=\text{O}$ bond); 1532 cm^{-1} (urethane amide II); 1081 cm^{-1} ($\text{C}(\text{O})-\text{O}-\text{C}$ stretching of the hard segment; and 821 cm^{-1} (bending vibration in benzene ring) (Khan et al. 2008; Tijing et al. 2012). The typical absorption bands for PEO are 2894 cm^{-1} (CH_2 stretching), $\text{C}-\text{O}-\text{C}$ stretching vibration at 1153 , 1101 , and 963 cm^{-1} , and at 1343 cm^{-1} assigned as $\text{C}-\text{H}$ deformation mode (Zhou et al. 2011). All peaks of neat PU were detected in the hybrid mats, and the additional peaks in Figure 3a are attributed to the peaks of PEO. However, the band at 1478 ($-\text{C}-\text{H}$ bending mode) of neat PU was observed to have shifted to lower frequency in the hybrid mats, indicating the formation of stronger hydrogen bond between PU and PEO (Pant et al. 2010). These FTIR spectra results confirm the successful fabrication of bicomponent hybrid nanofibrous mat consisting of PU and PEO nanofiber matrices.

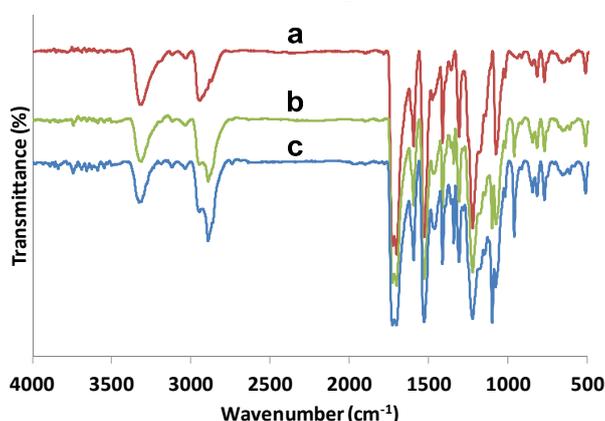


Fig. 3. FTIR spectra of: (a) neat PU mat; (b) PU-Ag/PEO (3 h), and; (c) PU-Ag/PEO (5.5 h) composite mats.

Figure 4 shows the antibacterial activity of the electrospun nanofibrous mats via a zone inhibition test. It was reported that Ag NPs with sizes of 1-10 nm can disturb the permeability of bacteria and respiratory functions, thus can efficiently inactivate them (Morones et al. 2005; Chen et al. 2008). In this study, Hybrid 1 nanofibrous mat, which contains Ag NPs with average diameter of 8 nm (Figure 4b) showed much higher bacterial inactivation as compared to Hybrid 2 (Ag NP average diameter = 90 nm) (not

shown), while individual nanofibrous mats of neat PU (Figure 4a) and neat PEO (not shown) alone did not show any signs of antibacterial activity. This suggests that without the aid of Ag NPs, the individual neat nanofibrous components of PU and PEO could not provide any antibacterial effect. The higher bacterial activity of Hybrid 1 could be attributed to the presence of smaller Ag NPs, which provide large surface area to come in contact with bacteria, when compared with bigger Ag NPs in Hybrid 2 (Pal et al. 2007).

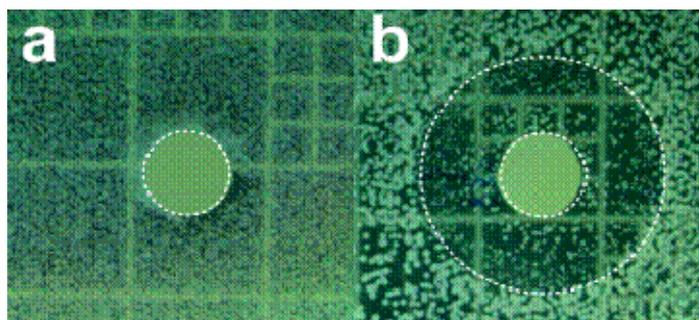


Fig. 4. Zone of inhibition against *E. coli* of: (a) neat PU mat; and (b) PU-Ag/PEO (3 h).

SUMMARY

In summary, we have demonstrated a facile way to fabricate hybrid nanofibrous mats with antibacterial functionality by dual-spinneret electrospinning. Using PEO both as reducing agent of AgNO_3 and template for the formed Ag NPs, we can utilize the dual-spinneret electrospinning to selectively functionalized the component nanofibers for making new materials. Here, the larger PU nanofibers give structural stability and prolonged use of the hybrid materials, while the thinner PEO nanofibers provide more reactive surface area, and the decoration of Ag NPs on PEO surfaces without the use of chemicals gives antibacterial functionality to the hybrid mat. The reduction time of AgNO_3 in PEO plays an important role in the size control of the Ag NPs. The hybrid mat with Ag NPs of 8 nm average size (i.e., reduction time of 3 h) exhibited strong antibacterial activity. The present hybrid mat has great potential for antibacterial tissue scaffolds or for wound-healing applications.

ACKNOWLEDGEMENTS

This research was supported by a fund from Chonbuk National University Grant Fellow Project 2012, and by a Grant from the Regional Research and Development Cluster Project (B0009719) funded by the Ministry of Knowledge Economy of Korea, and also partially by a Grant from the Ministry of Education, Science and Technology of Korea through the National Research Foundation (Project no. 2012-013341).

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