

Synthesis, characterization, and antibacterial performance of Ag-modified graphene oxide reinforced electrospun polyurethane nanofibers

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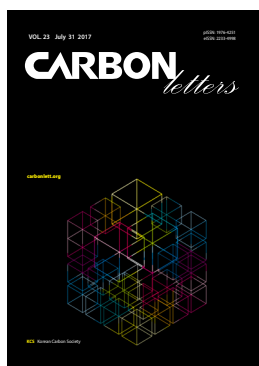
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Abstract

Polyurethane (PU) nanofibers containing graphene oxide (GO) and Ag doped functionalized reduced graphene oxide (Ag-RGO) were successfully prepared via the electrospinning technique. The uniform distribution of GO sheets along with Ag nanoparticle in the nanofibers was investigated by scanning electron microscopy and the elemental mapping technique. X-ray diffraction and thermal gravimetric analysis verified the presence of GO and Ag in the bicomposite nanofibrous mats. Antibacterial tests against *Escherichia coli* demonstrated that the addition of GO and Ag-RGO to the PU nanofiber greatly enhanced bactericidal efficiency. Overall, these features of the synthesized nanofibers make them a promising candidate material in the biomedical field for applications such as tissue engineering, wound healing, and drug delivery systems.

Key words: electrospinning, Ag-RGO, nanocomposite, antibacterial, *Escherichia coli*

1. Introduction

In recent years, polymer electrospinning techniques have been increasingly investigated. Electrospinning is considered a simple and easy method for controlling the morphology of ultrafine fibers. Different types of polymers as well as blends of other polymers/oligomers have been successfully electrospun into micro/nanofiber matrices for various applications [1]. The fibers produced by this method have shown amazing characteristics, including very large surface-to-volume ratios, and high porosity with small pore size [2,3]. These characteristics make the electrospun fibers suitable for many potential applications such as filtration, protective clothing, tissue scaffold, wound dressing, drug delivery systems, sensors, and optical materials [4-7].

Recently, graphene has attracted widespread attention because of its remarkable properties which include superior mechanical strength [8], excellent mobility of charge carriers [9], high thermal conductivity [10], and large specific surface area [11]. The integration of graphene into materials such as metal, metal oxides, and polymers could endow them with unique functionalities [12]. However, the inherent absence of functional groups on the graphene sheets limits their integration with polymers and metal/metal oxides. Moreover, the homogeneous dispersion of graphene in a polymer matrix remains challenging due to its hydrophobic nature and incompatibility with organic polymer.

To solve this problem, graphene oxide (GO) was developed as an alternative to pristine graphene. GO is synthesized by introducing a large number of hydrophilic functional groups on its surface [13-15]. Moreover, compared to other carbon materials such as graphene, carbon nanotubes, etc., GO exhibits good dispersibility in polymer solutions, and

does not strongly hinder the spinnability of polymer solution. At the same time it provides sufficient sites for polymeric molecules to interact with it by the formation of hydrogen bonds [16]. Accordingly, GO can be considered one of the most important derivatives of graphene.

Silver nanoparticles (NPs) exhibit excellent antibacterial activity without the release of toxic biocides, and as a result Ag NPs are considered non-toxic and environmentally friendly materials in biomedical applications [13,15,17]. To be effective, polymeric materials filled with Ag NPs have to release the Ag ions into a pathogenic environment continuously [17,18]. Recently, electrospun polymer nanofibers with embedded Ag NPs have attracted considerable attention mainly due to their antimicrobial activities. Considering the separate attractive merits of GO and Ag NPs, and the intriguing properties of polyurethane (PU) nanofibers, composites of these materials could have novel properties as biomaterials. Consequently, in this study, we prepared a hybrid mat of PU and functionalized GO containing Ag NPs, using a simple electrospinning technique. The resulting materials demonstrated excellent antibacterial performances, recommending their use for the biomedical applications.

2. Experimental

2.1. Materials

Synthetic graphite from Aldrich (average particle diameter $<20\ \mu\text{m}$) was used to prepare the GO. Potassium permanganate, hydrogen peroxide, sulfuric acid, hydrazine monohydrate, 1, 4 aminophenol, and silver nitrate were obtained from Sigma-Aldrich (USA). PU (Mw=110,000) was purchased from Cardio Tech. Intern., Japan. Tetrahydrofuran (THF) and N,N dimethylformamide (DMF) were purchased from Showa (Japan) and used as solvents. All of the chemicals were of analytical grade and used without further purification.

2.2. Preparation of GO

GO nanosheets were synthesized using the modified Hummer's method [19]. In a typical procedure, 125 mL of concentrated sulfuric acid was taken into 500-mL three necked round bottom flask filled with graphite powder (5 g) followed

by the addition of solid potassium permanganate (17.5 g) slowly at 0°C (ice bath). This mixture was agitated by Teflon coated stirrer for 3 h at 35°C and then diluted by adding a sufficient amount of distilled water at 0°C (ice bath). H_2O_2 (30 vol% in water) was added until the bubbling of the gas was completed. The product, obtained in this way, was made free from acid by centrifuging and washing several times with deionized water and subsequently dried under vacuum at 70°C for 2 d. The desired brown powder of graphite oxide was obtained.

2.3. Preparation of Ag doped functionalized reduced GO nanocomposite

Aminophenol functionalized and Ag NPs decorated GO sheets were prepared in accordance with our previous report [13]. Briefly, 1.5 g of GO was dispersed in 500 mL of THF by magnetic stirring for 1 d followed by ultrasonication for 1.5 h at 250 W. In a separate step, a dispersion of aminophenol in ethanol was prepared by completely dissolving 6 g of aminophenol in 400 mL ethanol. Both solutions were mixed together and a homogeneous mixture was formed after 2 h ultrasonication. Then the dilute solution of AgNO_3 in ethanol (1.7 g solid content) was added to the above mixture. The resulting mixture was agitated at 60°C for 5 h. After several times washing with water and ethanol, it was dried in an oven at 70°C for 12 h.

2.4. Preparation of Ag doped functionalized reduced GO/PU composite nanofiber mat

The neat PU solution was prepared by dissolving the PU in a DMF/THF (1:1) solvent solution. Electrospinning was carried out at 15 kV while maintaining a tip-to-collector distance of 15 cm. To prepare PU nanofibers containing GO, and Ag-FGO, 2 wt% of GO and Ag-FGO suspensions in DMF were added separately into the above solution (the final concentration of the PU solution was 10 wt%) and the electrospinning was carried out under identical conditions. The prepared nanofiber mats were then collected on aluminium foil and vacuum dried for 12 h at 60°C , and finally used for characterization. A schematic diagram representing the preparation of the Ag doped functionalized reduced GO (Ag-RGO) decorated PU nanofibers is given in Fig. 1.

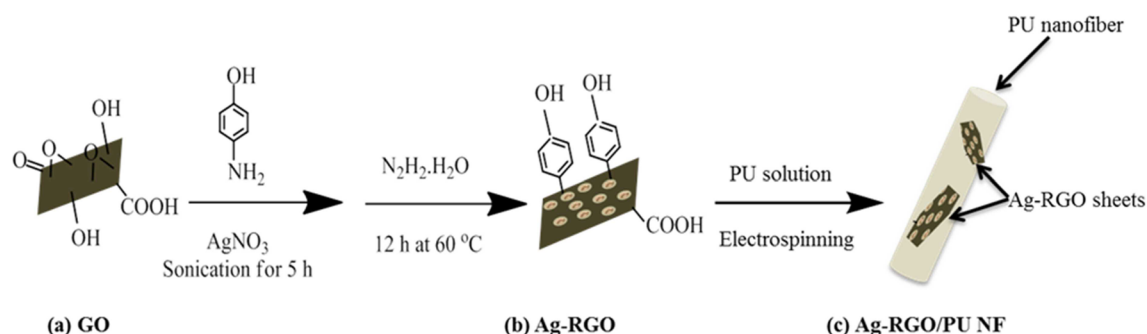


Fig. 1. Schematic illustration of the fabrication of the Ag-RGO/PU NF. GO, graphene oxide; Ag-RGO, Ag doped functionalized reduced graphene oxide; PU, polyurethane; NF, nanofiber.

2.5. Characterization

The surface morphology of the nanofibers was studied using a Jeol JSM-5900 scanning electron microscope (JEOL, Japan). The phase and crystallinity were characterized with a Rigaku X-ray diffractometer (Rigaku, Japan) with Cu K α ($\lambda=1.54056$ Å) radiation over a 2θ range of angles, from 10° to 80° . The thermal property of the nanofiber mats was studied by thermal gravimetric analysis (PerkinElmer, USA).

2.6. Antibacterial performance

The antibacterial performance of the different as-synthesized nanofiber membranes was investigated by a colony count method using *Escherichia coli* as the model microorganism, as in our previous experiments [13,17]. Briefly, the bacterial inoculum was prepared in tryptic soy broth containing 17 g/L pancreatic digest of casein, 3 g/L dipotassium phosphate to give a bacterial concentration of about 4.5×10^8 CFU/mL. Then, the bacterial suspension was incubated at 37°C for 12 h with shaking. One hundred microliter from the stock solution was spread on a luria bertani (LB) agar plate containing electrospun mats followed by incubation at 37°C for 12 h. Then all the agar plates were visually inspected for the presence of bacterial growth, and the results were recorded.

3. Results and Discussion

Fig. 2 shows the morphology of the as-synthesized nanofibers. The pristine PU nanofiber has a uniform and continuous morphology (Fig. 2a). It was observed that the rate of nanofiber production increased when we used GO along with the polymer solution. The presence of GO in the solution increases conductivity and decreases viscosity [16] which accelerates the jetting of polymer during the electrospinning process. Therefore, the

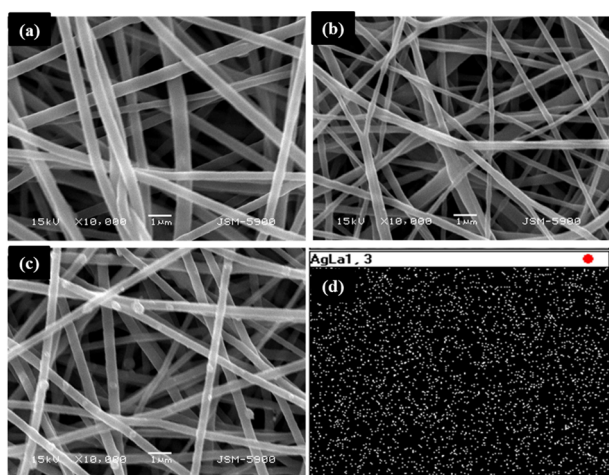


Fig. 2. Scanning electron microscopy images of pristine PU nanofiber (a), GO-PU nanofiber (b), and Ag-RGO/PU nanofibers (c). (d) The elemental mapping image of (c), showing the distribution of the Ag NPs. PU, polyurethane; GO, graphene oxide; Ag-RGO, Ag doped functionalized reduced graphene oxide.

highly conductive polymer solution achieved a faster speed during electrospinning. We subsequently observed that the diameter of the nanofibers after the addition of GO were reduced as compared to the pristine PU nanofibers.

In the case of the Ag-RGO/PU nanofiber, GO sheets along with Ag NPs attached on the surface of PU nanofibers can be seen (Fig. 2c). The elemental mapping image (Fig. 2d) confirms the uniform distribution of the Ag NPs throughout the nanofiber.

The deposition of Ag NPs along with GO in the PU matrix was also confirmed by X-ray diffraction (Fig. 3). The PU nanofiber membrane shows a peak around 20° similar to reports in the literature [20]. The peak of GO was observed at 2θ value of 11.5° in the case of the GO/PU nanofiber membrane [13]. Extra peaks were observed at 2θ values of 38.2° , 44.3° , 64.4° , and 77.5° corresponding to the crystal planes (111), (200), (220), and (311), respectively indicating the presence of Ag NPs in the composite mats [13,15,17] of the Ag-RGO/PU. Moreover, the disappearance of the GO peak at 2θ value of 11.5° indicated that the GO was significantly reduced to RGO, and had been well incorporated inside the fiber matrix.

In order to investigate the thermal stability and the composition of the prepared catalyst, thermogravimetric analysis was carried out in air at temperatures from 30°C to 800°C . Fig. 4 shows the thermal gravimetric analysis profiles of the different formulations. For the pristine PU sample, the initial decomposition temperature is about 290°C . From the curves, it can be noted that the composite nanofiber mats display single-stage thermal degradation; however, the onset decomposition temperature for each sample was observed to be different. A similar trend in weight loss can be seen in the case of composite samples; however, the residual weight fractions were found to be higher than that of a pristine sample, which confirms the content of GO and Ag-RGO in the composite materials.

The antibacterial properties of the PU nanofibers containing GO and GO-Ag NPs were tested on Gram-negative *E. coli* microorganisms as explained in the experimental section. The results for the GO-PU and Ag-RGO/PU nanocomposite mats were

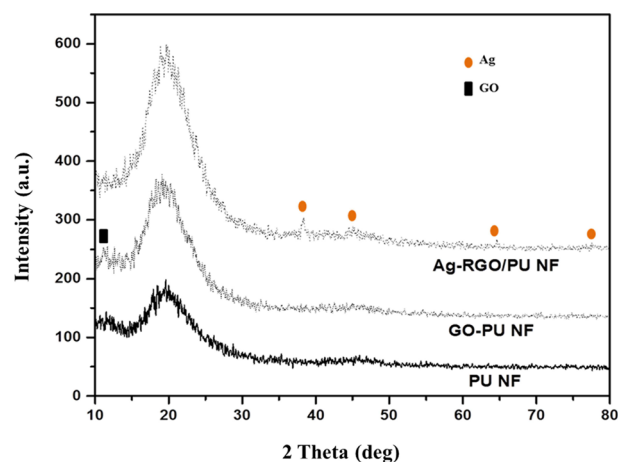


Fig. 3. X-ray diffraction spectra of pristine PU NFs, GO-PU NFs, and Ag-RGO/PU composite nanofibers. GO, graphene oxide; Ag-RGO, Ag doped functionalized reduced graphene oxide; PU, polyurethane; NF, nanofiber; deg, degree.

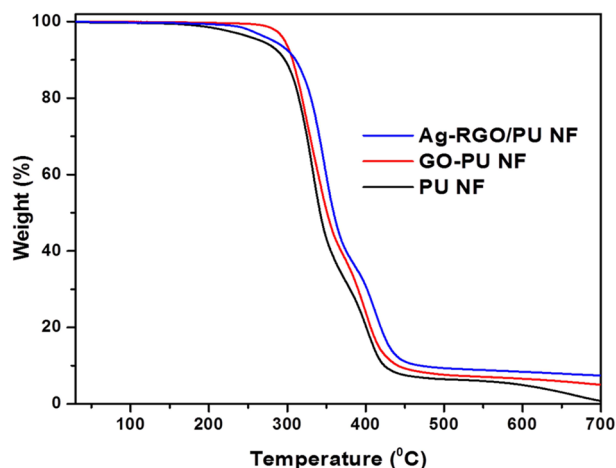


Fig. 4. Thermogravimetric analysis of pristine PU NFs, GO-PU NFs, and Ag-RGO/PU composite nanofiber mats. Ag-RGO, Ag doped functionalized reduced graphene oxide; PU, polyurethane; NF, nanofiber; GO, graphene oxide.

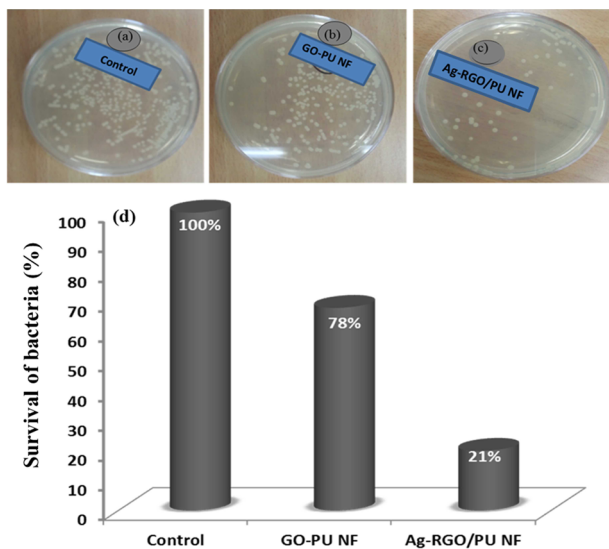


Fig. 5. Antibacterial performances of different mats; Control (a), GO-PU NFs (b), and Ag-RGO/PU (c) against *Escherichia coli* bacteria by the colony count method and the viability of *E. coli* after 8 h of treatment on different samples (d). GO, graphene oxide; PU, polyurethane; NF, nanofiber; Ag-RGO, Ag doped functionalized reduced graphene oxide.

compared with those of the pristine PU mat.

From Fig. 5, it can be seen that the pure PU nanofibers showed no antibacterial activity. In contrast, the GO and Ag-RGO NPs decorated PU nanofibers showed excellent bactericidal efficiency to *E. coli*; however, this effect was more pronounced in the case of the Ag-RGO/PU nanofibers mat. As shown in Fig. 5b, ~79% of the bacteria were killed by the nanocomposite membrane after 12 h of incubation.

The antibacterial action of GO against bacterial cells is due to the generation of reactive oxygen species which leads to cell death [13,15]. As compared to GO, Ag is a strong antibacterial

agent. Ag NPs can anchor to the bacterial cell wall and subsequently penetrate it, thereby causing structural changes in the cell membrane and death of the cell [21].

Previous studies have also shown that the nanofiber mats immersed into aqueous solution gradually release Ag⁺ ions or Ag NPs [17]. The formation of free radicals by the Ag NPs is considered to be another mechanism by which the cells die. Moreover, Ag NPs can interact with the sulfur and phosphorus in DNA, leading to problems in the DNA replication of the bacteria, and thus terminates the microbes [22]. Furthermore, the larger surface area in smaller Ag NPs may increase the opportunity for interaction with the bacterial surface and promote a stronger antibacterial effect. Thus, it can be concluded that the excellent antibacterial activity shown by the Ag-RGO/PU mat is due to the combined effects of the Ag and GO.

4. Conclusions

The assembly of GO and Ag-RGO on the surface of PU nanofibers via a one-step electrospinning process was successfully demonstrated. The method features the novel strategy of using the electrospinning process to incorporate the GO and metal nanoparticles into the nanofibers. The synthesized nanofiber mat decorated with Ag-RGO exhibited very strong antibacterial activities against *E. coli*. Accordingly, it is anticipated that the obtained PU nanofibers decorated with RGO and Ag NPs can be used in various applications such as wound dressing, biofilm, bioadhesive, and the coating of biomedical materials.

Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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