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Facile preparation and characterization of poly(vinyl alcohol)/chitosan/graphene oxide biocomposite nanofibers

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ABSTRACT

Poly(vinyl alcohol) (PVA)/chitosan (CS)/graphene oxide (GO) biocomposite nanofibers have been successfully prepared using aqueous solution by electrospinning. CS colloidal gel in 1% acetic acid can be changed to homogeneous solution by using electron beam irradiation (EBI). The uniform distributions of GO sheets in the nanofibers were investigated by field emission scanning electron microscopy (FESEM) and Raman spectroscopy. FESEM images illustrated that the spread single GO sheet embedding into nanofibers was formed via self-assembly of GO sheet and PVA/CS chains. And the average diameters of the biocomposite nanofibers decreased (200, 173, 160 and 123 nm) with increasing the contents of GO (0.05, 0.2, 0.4 and 0.6 wt%). Raman spectra verified the presence of GO in the biocomposite nanofibrous mats. The mechanical properties of as-prepared materials related with GO contents. It revealed that the highest tensile strength was 2.78 MPa, which was 25% higher than that of neat PVA/CS nanofibers. Antibacterial test demonstrated that the addition of GO to PVA/CS nanofiber had great ability to increase inhibition zone till 8.6 mm. Overall, these features of PVA/CS/GO nanofibers which were prepared by eco-friendly solvent can be a promising candidate material in tissue engineering, wound healing and drug delivery system.

1. Introduction

Graphene, unique two-dimensional single or few layers of sp^2-bonded carbon sheet, has attracted great research interesting because of its exceptional physical and chemical properties that leads to many potential applications, such as senor [1], energy technology [2], catalysis [3], electronics [4], and so on [5]. In recent years, interest in graphene and its derivatives as biomedical materials is a relatively new field with significant potential [6,7]. GO is an oxidized form of graphene which usually produced by oxidizing graphite, while phenol hydroxyl and epoxide groups are introduced to the basal plane and carboxylic groups at the edges. GO is soluble in water and some polar solvents because of these abundant oxygen-containing groups, and exhibits excellent biocompatibility. There is great potential for GO in biomedical applications such as tissue engineering and drug delivery. But graphene and its derivatives tend to aggregate in a layer-by-layer mode due to strong interplanar interactions, which means a considerable part of their surface area will be lost. It is a challenge to disperse single-layered GO as functional group with the polymer matrix, which allows one to utilize the surface area to the utmost extent. Thus, using appropriate solvent to dissolve the filler and the polymer matrix is the most important approach to fabricate polymer/GO nanofibers for biomaterials.

Recently, several polymer/GO composite nanofibers have been prepared. Pant et al. [8] successfully prepared nylon-6 spider-wave-like nano-nets of by adding suitable amounts of GO in polymer solution (4:1 wt% ratio of $CH_3COOH/HCOOH$) during electrospinning. In this case, GO sheet dispersed well in nylon-6 solution because of the formation of hydrogen bond between polymer molecules and GO sheet. Qi et al. [9] and Wang et al. [10] all fabricated PVA/GO nanofibers by electrospinning. The former

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dissolved powdery PVA to GO suspension aqueous solution to obtain a homogeneous suspension and the latter dispersed GO in PVA aqueous solution to form stable colloidal suspensions. They all took advantage of the functional groups of GO can form hydrogen bonds with the polymer matric molecule chains. However, as-prepared these nanofibers did not show higher surface utilization because of the curly GO sheets or fiber-covered GO, which can be improved by remaining the functional surface of expanded single-sheet GO.

CS-based nanofibers have been identified as an excellent biomaterial, especially as tissue scaffolds or wound healing material. However, CS is extremely difficult to electrospun due to its low solubility in common organic solvent and low stability. The published papers of neat CS or CS/polymer nanofibers [11–13] were all using the toxic solvents such as trifluoroacetic acid (TFA), TFA/dichloromethane (DCM) or acrylic acid (AA), which limited its application in biomedical field. In our past work, PVA/CS nanofibers in 1% acetic acid aqueous solution were successfully prepared by combine electron-beam irradiation (EBI) and electrospinning technique. EBI have been used to accomplish not only cross-linking [14] but also diminishing molecular weight such as polysaccharides [15] without the use of cross-linkers and toxic reagents. The molecular weight of CS can be reduced without changing the main structure at the irradiation dose of 50 kGy. The advantage of this strategy lied on non-toxic and environmentally friendly aqueous solution.

However, the properties of PVA/CS nanofibers can be improved greatly, such as mechanical tension, antibacterial property and so on. In this paper, there were advanced materials of PVA/CS nanofibers with eco-friendly solution by adding GO as the fillers because of its biocompatibility, antibacterial activity and promotion in cell's attachment, proliferation and differentiation [9,16]. It is expected to obtain a kind of materials which would combine the advantages of PVA/CS composites nanofibrous and GO functional structure together. To the best of our knowledge, this is the first to fabricate CS/GO-based nanofibers with non-toxic solution. GO suspension was added into PVA/CS system to form stable homogeneous suspension, then electrospun to prepare PVA/CS/GO nanofibers and characterized their microstructure. Moreover, hydrophilic, mechanical and antibacterial tests were investigated the properties changes of the nanofibers with GO filler contents.

2. Experimental

2.1. Materials

Graphite flakes (325 mesh, 99.8%) were purchased from Johnson Matthey Co. Potassium permanganate ($KMnO_4$, 99.0%), PVA (Mw = 85,000–124,000, 87–89% hydrolyzed), CS (200,000 cps, the degree of deacetylation: 75–85%) and glyoxal solution (40 wt% in H_2O) were obtained from Aldrich Co., USA. Sulfuric acid (H_2SO_4, 97%), hydrogen peroxide (H_2O_2, 30 wt%), acetic acid and phosphoric acid were obtained from Samchun Pure Chemical Co., Ltd, South Korea. All the chemicals were used without further purification.

2.2. Synthesis of GO

GO was synthesized by a modified Hummers method [17]. Firstly, 4 g graphite flakes were added to a 250 mL flask containing 120 mL H_2SO_4 with stirring for 1 h. 15 g $KMnO_4$ was added to the mixture at intervals of 20 min by 9 times gradually. The mixture was slowly heated to 40 °C with keeping it for 5 h in order to oxidize the graphite. Secondly, 150 mL of deionized (DI) water was added to the system. A 15 mL H_2O_2 solution was added to the mixture under stirring for 30 min and then remained in this condition for 24 h and centrifuged. After that, the mixture deposed in the dialysis tube was washed with DI water several times to obtain a pH of 5. Finally, the GO was dried in a freeze dryer at −45 °C for 24 h.

2.3. Preparation of PVA/CS-GO suspensions and electrospinning

Briefly, non-precipitate and brown solution of 8 wt% CS was prepared after EBI treatment. 20 wt% PVA solution was prepared by dissolving PVA powder in DI water at 90 °C for 24 h. The homogeneous aqueous solution was obtained by mixing 2 g PVA and 5 g CS solution under vigorously magnetic stirrer. Then glyoxal solution (6 wt% with PVA) was added as cross-linking agent while the pH of the system was adjusted at 2–3 by phosphoric acid. Furthermore, dried GO was dispersed in DI water to suspensions (20 mg/ml) with the help of mild sonication for 1 h, then was added to the PVA/CS system by different volumes (0.2, 0.8, 1.6 and 3.0 ml) and continually stirring for 2 h. The PVA/CS-GO suspensions with various contents (0.05, 0.2, 0.4, 0.6 wt%) solutions were electrospun at 18 kV by maintaining a tip-to-collector distance of 16 cm. As-spun PVA/CS/GO nanofibers were collected in Teflon paper; put into the oven under alcohol vapor at 50 °C for 24 h, and then cured at 120 °C for 10 min. Procedure of PVA/CS nanofibers with self-assembled GO is shown in Fig. 1.

2.4. Morphological and structural characterizations

The chemical structure of GO powder and PVA/CS/GO mats were confirmed by Fourier transform infrared spectroscopy (FT-IR, Varian 1000 FT-IR Scimitar series, PIKE technologies, USA). The surface morphologies and diameters were determined with Field emission scanning electron microscopy (FESEM, S-7400, Hitachi, Japan). Raman spectra were obtained using FT-Raman spectroscopy (RFS-100S, Bruker, Germany).

2.5. Contact angle characterizations

Information about wettability was obtained by the deionized water contact angle measurement using contact angle meter (Digidrop, GBX, France). Deionized water was automatically dropped onto the electrospun mats.

2.6. Mechanical properties characterizations

Mechanical properties were measured with a universal testing machine (AG-5000G, Shimadzu, Japan), under a crosshead speed of 5 mm/min at room temperature. The samples were prepared in the form of standard dumbbell shapes according to ASTM Standard D 638 via die cutting from the electrospun mats and tested in the machine direction. At five measurements were performed for each sample.

2.7. Antibacterial properties characterizations

The antibacterial activities of PVA/CS nanofibers membranes with and without GO were investigated by a zone inhibition method. *Escherichia coli* (*E. coli*) cells were used as the model microorganisms. One colony of *E. coli* was taken out from the original stock in an agar plate, centrifuged at 200 rpm/min and was cultured lysogeny broth (LB) medium. All cells were grown overnight in LB medium at 37 °C for 24 h. The CS/PVA nanofibers composite nanofibers with different contents of GO were cut into 6 mm diameter discs. Then, nanofibers discs were transfered to *E. coli* bacterium inoculated plates. The PVA/CS/GO nanofibers composite nanofibers were kept at uniform distance and then incubated over-night at 37 °C. The zone of inhibition was observed after 24 h of incubation and the diameter of the zone was measured.