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ORIGINAL PAPER

Fabrication of ultrafine fibers of poly(γ-glutamic acid) and its derivative by electrospinning

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Summary Poly(γ-glutamic acid) (PGA) was successfully electrospun by the addition of poly(ethylene glycol) (PEG) and Triton X-100 in its aqueous solution to produce the PGA non-woven mat of the ultrafine fibers. The average fiber diameter was in the range between 200 nm and 2 μm. The fiber mat was quickly soluble in water due to the large surface area of the fibers. The electrospinning of PGA butyl ester with the esterification degree of 61% in 1,1,1,3,3,3-hexafluoro-2-propanol gave the water-insoluble nanofiber mat.

Keywords Poly(γ-glutamic acid) · Fiber · Electrospinning

Introduction

Electrospinning is a simple and cost-effective process to fabricate non-woven mats of ultrafine fibers in the diameter range from tens of nanometers to few microns through the actions of electrostatic forces [1–3]. Such electrospun non-woven mats

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exhibit large surface area and high porosity with very small pore size; thus they can be useful for scaffolds for tissue engineering [4, 5], matrices of controlled drug release [6, 7], dressings for wound healing [8, 9], medical implants [10], nanocomposites for dental applications [11], molecular separation biosensors, and preservation of bioactive agents [12].

There have been many studies on the fabrication of electrospun ultrafine fibers of biopolymers and their biomedical applications utilizing their good biocompatibility and biodegradability, high hydrophilicity, less-immune reaction as well as enhanced cell adhesion and proliferation [1–3]. So far, the electrospinning of various biopolymers such as collagen [8, 13, 14], gelatin [5, 15–17], hyaluronan [16–18], chitin [19], chitosan [20–23], cellulose [24, 25], and alginate [26, 27] were reported. Some of these biopolymers themselves could not be electrospun. In such a case, the addition of poly(ethylene glycol) (PEG) was often effective for the fabrication of fiber mats [27, 28].

Poly(γ-glutamic acid) (PGA) is a bio-product secreted by *Bacillus subtilis*. PGA is substantially biodegradable, nontoxic to humans, and even edible; thus, its industrial applications have been extensively studied from industrial standpoints in past years [29]. Its multi-functionality, biodegradability, nontoxicity, compatibility, and edibility, have made it a promising biopolymer for various uses such as health foods, moisturizers in cosmetics, chelating agents in waste-water treatment, hydrogels for environmental, agricultural, and biomedical product applications, and biodegradable packing materials.

This study deals with fabrication of ultrafine fibers of PGA and its derivative by electrospinning. This is the first example of electrospinning of PGA to fabricate the non-woven mat useful for various bio-related applications.

Experimental

Materials

PGAs (sodium form, Mw = 2×10^3, 5×10^3, and 7×10^3 kDa) were products of Bioleaders Corp. (Korea). PEGs (Mw = 5×10^2, 1×10^3, 4×10^3, and 8×10^3 kDa) were purchased from Sigma (USA). Other reagents and solvents were commercially available and used as received.

Synthesis of PGA butyl ester

Butyl ester of PGA (Mw = 2×10^3 kDa) was prepared according to the literature [30, 31]. PGA (0.39 g, 3.0 mmol of monomer unit) was dissolved in 20 mL of DMSO, and sodium hydrogen carbonate (0.76 g, 9.0 mmol) and bromobutane (1.2 g, 9.0 mmol) were added to the solution and kept under gentle stirring at 60 °C for 1 day. The insoluble part was removed by the filtration, and the filtrate was poured into a large amount of toluene. The precipitate was collected by the filtration, followed by washing with methanol. The residue was dispersed in water and the impurity was removed by using cellulose membrane tube (cut off molecular

weight of 2×10^3) with 7 times exchanging water for 1 week. The product was isolated by lyophilization in 86% yield. ^{1}H NMR (DMSO–d_6): δ 0.9 (t, CH_3), 1.3 (m, CH_3–$C\underline{H}_2$), 1.5 (m, CH_3CH_2–$C\underline{H}_2$) 1.7, 1.9 (br, β-CH_2 of PGA), 2.2 (br, α-CH_2 of PGA), 4.1 (t, O–CH_2), 4.2 (br, γ-CH of PGA) and 8.3 (br, NH).

Electrospinning

Electrospinning of PGA was carried out as follows. A mixed aqueous solution of PGA, PEG, and Triton X-100 was placed in a 10 mL of a glass syringe with a 18 gauge needle, which was connected to a high voltage generator. The glass syringe was horizontally mounted in a syringe pump. The flow rate of the delivery system and the distance between the needle tip and the drum collector were fixed as 1 mL/min and 10 cm, respectively. The grounded rotating metal drum served as a counter electrode. A voltage of 20 kV was applied to the solution and the jet emerging from the solution was collected on the drum. The surface morphology of the resultant fiber mat was observed by scanning electron microscopy (SEM).

For the electrospinning of PGA butyl ester, 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) was used as solvent and the flow rate of the delivery system was fixed as 2 mL/min. Other conditions were the same as those of PGA.

Measurements

NMR spectra were recorded on a Bruker DPX400 spectrometer. SEM measurement was carried out by using Hitachi S-3000N with an acceleration voltage of 20 kV.

Results and discussion

At first, the aqueous solution of PGA (Mw = 2×10^3 kD) with various concentrations was electrospun, but no fiber formation was observed. Thus, PEG and Triton X-100 were added to the PGA solution and the electrospinning of the mixed solution was examined. This combination afforded the mat of PGA ultrafine fibers. In this study, the concentration of Triton X-100 was fixed as 1.3%.

For the initial trial of the electrospinning, PEG with molecular weight of 4×10^3 kDa was used and the weight ratio of PGA:PEG was fixed as 70:30. Figure 1 shows SEM images of the surface morphology of the mat obtained by the electrospinning with the different concentration of PGA. In all cases, the fiber formation was observed. Below the PGA concentration of 6%, the clear image of the fibers was not obtained and the formation of a small amount of beads was found. The 8% PGA solution yielded the ultrafine cylindrical fibers in the diameter range from 300 to 700 nm. In case of the 10% PGA solution, micron-size fibers were formed. These data show that the addition of PEG and Triton X-100 gave the PGA ultrafine fibers, and the fiber morphology and diameter strongly depended on the PGA concentration.

The effect of the molecular weight of PEG on the fiber formation was examined (Fig. 2). The electrospinning conditions were as follows: PGA:PEG = 70:30 or

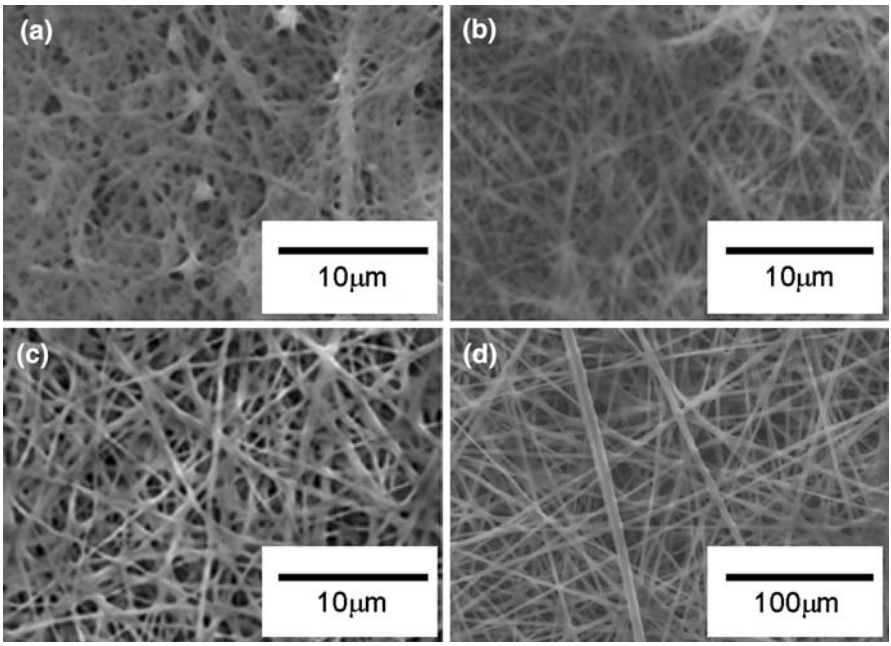

Fig. 1 SEM images of the electrospun fibers obtained from the PGA/PEG mixed solution (PGA:PEG = 70:30) with PGA concentration of **a** 4%, **b** 6%, **c** 8%, and **d** 10%

80:20; PGA concentration = 10%. In case of the PGA content of 70%, the micron-size fibers were formed, whereas the 80% PGA afforded the nanofibers of PGA. The fiber diameter slightly increased as a function of the PEG molecular weight. In using PEG with the molecular weight of 8×10^3 kD, the clear image of the fibers was not obtained, probably due to the high viscosity of PEG. The uniform fibers were not formed in the PGA content of 90% (data not shown).

The effect of the molecular weight of PGA was examined (Fig. 3). The electrospinning conditions were as follows: PGA:PEG = 80:20; PGA concentration = 10%, PEG molecular weight = 5×10^2 kD. The formation of ultrafine fibers from PGAs with molecular weight of 2×10^3, 5×10^3, and 7×10^3 kDa was observed and the molecular weight of PGA scarcely affected the fiber diameter.

Although the powder of the sodium form of PGA is soluble in water, the vigorous mixing is required for the solubilization; several hours are mostly necessary for preparation of the aqueous high concentration solution of the high molecular weight PGA. On the other hand, the resulting PGA ultrafine fiber mat was readily soluble in water; by immersion into water, the mat completely disappeared only in a few minutes. This may be due to the large surface area of the ultrafine fiber mat of PGA. This unique property of the PGA mat may be useful for applications such as foods and cosmetics.

Next, the water-insoluble mat of ultrafine fibers of PGA was fabricated by electrospinning of PGA butyl ester. The esterification degree determined by ^{1}H NMR was 61%. The electrospinning of PGA butyl ester with the different

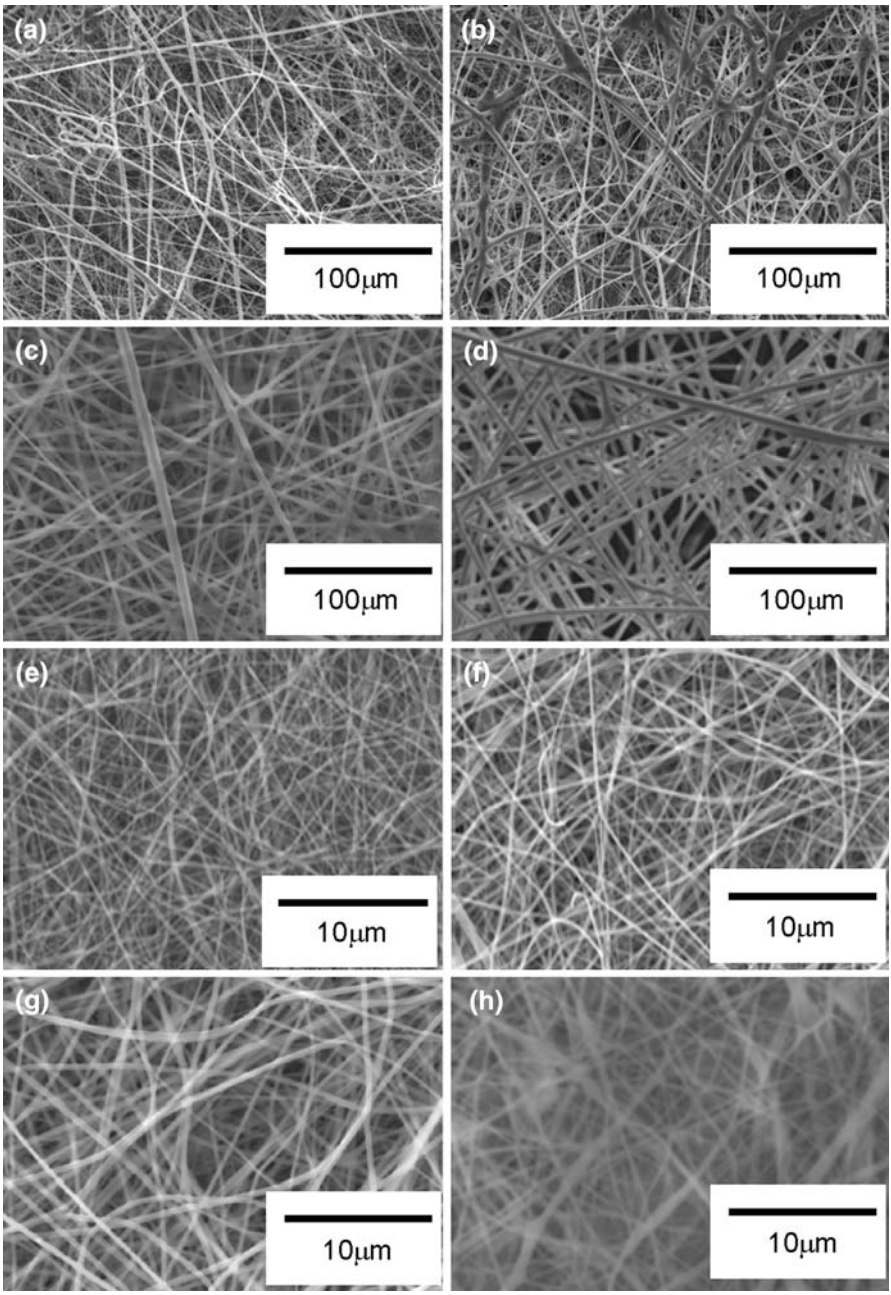

Fig. 2 SEM images of the electrospun fibers obtained from the PGA/PEG mixed solution [PGA concentration = 10%, (**a**–**d**) PGA:PEG = 70:30, (**e**–**h**) PGA:PEG = 80:20] with PEG molecular weight of (**a**, **e**) 5×10^2 kDa, (**b**, **f**) 1×10^3 kDa, (**c**, **g**) 4×10^3 kDa, and (**d**, **h**) 8×10^3 kDa

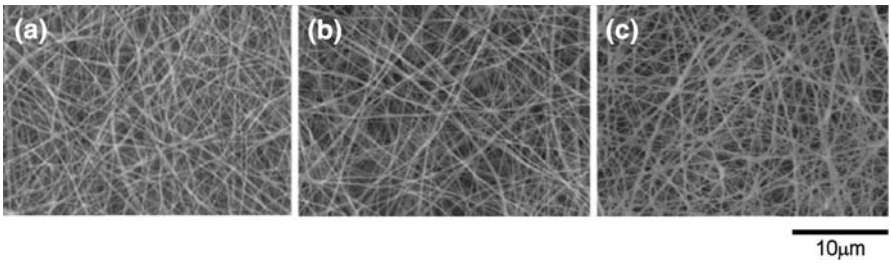

Fig. 3 SEM images of the electrospun fibers obtained from the PGA/PEG mixed solution (PGA:PEG = 80:20) with PGA molecular weight of **a** 2 × 10^3 kDa, **b** 5 × 10^3 kDa, and **c** 7 × 10^3 kDa

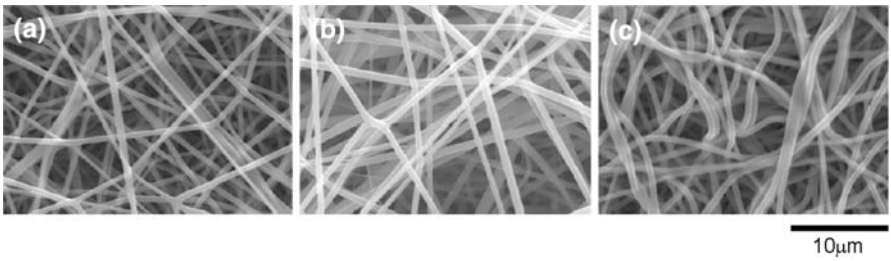

Fig. 4 SEM images of the electrospun fibers obtained from the PGA butyl ester with concentration of **a** 6%, **b** 8%, and **c** those after immersion in deionized water for 1 day

concentration was carried out by using HFIP as solvent (Fig. 4). Below the concentration of 4%, the clear image of the fibers was not obtained (data not shown). In the concentration of 6 and 8%, cylindrical ultrafine fibers in the diameter range from 200 to 700 nm were obtained. The resulting mat was insoluble in deionized water as well as phosphate buffer saline (pH 7.4), although the fiber morphology slightly changed.

Conclusions

Ultrafine fiber mats of PGA and PGA butyl ester were successfully fabricated by electrospinning. PGA itself was not electrospun under any spinning conditions, however, the addition of PEG and Triton X-100 afforded the PGA fiber mat in the diameter range from 200 nm to 2 μm. The fiber formation and diameter strongly depends on the PGA concentration, PEG molecular weight, and mixing ratio. The ultrafine fiber mat from PGA was readily soluble in deionized water, whereas the mat of PGA butyl ester was insoluble in deionized water. These PGA ultrafine fibers are expected for various applications in bio-related fields on the basis of unique properties of PGA.

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