Electrospinning of Poly(ethylene oxide) with Bacterial Cellulose Whiskers

*Won-Il Park, Minsung Kang, Hun-Sik Kim, Hyoung-Joon Jin**

Summary: In the present study, cellulose whiskers were incorporated into nanofibers of polyethylene oxide (PEO) by the electrospinning process to enhance the mechanical properties of the electrospun PEO fibers. Cellulose whiskers consisting of highly crystalline rod-like particles with a high aspect ratio and specific area were obtained by the acid hydrolysis of bacterial cellulose microfibrils. From the transmission electron microscopy (TEM) images of the bacterial cellulose whiskers, their length was measured to be 420 ± 190 nm and their width 11 ± 4 nm. The height of the whiskers was measured to be 10 ± 2 nm by atomic force microscopy. The successful formation of electrospun fibers with a diameter of less than 1 μm and well-embedded microfibrils was confirmed by scanning electron microscopy and TEM.

Keywords: bacterial cellulose; electrospinning; nanocomposite; nanofibers

Introduction

Electrospinning is a fast and simple process driven by the electrical forces on the surface of polymeric fluids, producing polymer filaments using an electrostatic force.[1–4] This electrospinning technique can serve various purposes, such as the fine control of the fiber diameters, the production of a defect-free or defect-controllable fiber surface, and the formation of continuous single nanofibers.[5] The outstanding properties of polymer nanofibers include their very large surface area, flexibility in terms of their surface functionalities, and good mechanical performance.[6] Polymeric nanofibers can be utilized in a wide range of applications such as texturing, filters, artificial tissue for membrane separation, porous electrodes, biomaterials, and composite reinforcement.[1–10]

In the present study, cellulose whiskers were incorporated into the nanofibers of polyethylene oxide (PEO) by the electrospinning process to enhance the mechanical properties of the PEO. The cellulose whiskers were obtained by the acid hydrolysis of bacterial cellulose microfibrils and consisted of highly crystalline rod-like particles with a high aspect ratio and specific area. *Acetobacter xylinum* is known to produce cellulose extracellularly. This bacterial cellulose is expected to become a new industrial material, because of its unique structure and properties in terms of its purity, high crystallinity, ultrafine network, high mechanical stability and low density.[11]

Experimental Part

Materials

Acetobacter xylinum BRC-5 was obtained from Yonsei University and used to produce the bacterial cellulose pellicles. The bacterium was cultured on Hestrin & Schramm (HS) medium, which was composed of 2% (w/v) glucose, 0.5% (w/v) yeast extract, 0.5% (w/v) bacto-peptone, 0.27% (w/v) disodium phosphate and 0.115% (w/v) citric acid. All of the cells were pre-cultured in a test tube containing a small cellulose pellicle on the surface of the medium and then inoculated into a 500 mL

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Erlenmeyer flask containing 100 ml of the HS medium. The flasks were incubated statically at 30 °C for 14 days. The cellulose pellicles were dipped into 0.25 M NaOH for 48 h at room temperature in order to eliminate the cells and components of the culture liquid. The pH was then lowered to 7.0 by repeated washing with distilled water. The purified cellulose pellicles were stored in distilled water at 4 °C to prevent them from drying. PEO with an average molecular weight of 9×10^5 (Aldrich) was used in the blending process.

Preparation of Hydrolyzed Bacterial Cellulose Fibrils

The bacterial cellulose fibrils were prepared by acid hydrolysis in 65 wt% sulfuric acid at 40 °C for 16 h with continuous stirring. After completion of the hydrolysis, the reaction was quenched by dipping the flask in a bath of ice-cold water. The cellulose whiskers were obtained by thorough washing using centrifugation and dialysis. The final concentration of the aqueous dispersions was generally 0.07 wt%, which was determined from the weight of the final solution.

Electrospinning

The bacterial cellulose fibril/PEO blends in water were prepared by adding PEO solutions to the aqueous bacterial cellulose solutions (5.0 wt%). Electrospinning was performed using a steel capillary tube with a 1.5 mm inside diameter tip mounted on an adjustable, electrically insulated stand, as described in an earlier study.[1] The capillary tube was maintained at a high electric potential for electrospinning and mounted in the parallel plate geometry. The capillary tube was connected to a syringe filled with 5 mL of a bacterial cellulose/PEO blend or PEO solution.

Characterization

The samples for transmission electron microscopy (TEM) were examined in a Philips CM 200 instrument. To examine the cellulose whiskers, a droplet of the diluted suspension was allowed to float on and eventually flow through a copper grid covered with a carbon film. The samples were then stained by allowing the grids to float in a 2 wt % solution of uranyl acetate for 3 min. The surface morphology of the samples was examined using a Hitachi S-4300 field emission scanning electron microscope (FESEM). The accelerating voltage applied for the cellulose whiskers and nanocomposites was 15 kV. To prepare the sample of cellulose whiskers, a droplet of the diluted suspension was allowed to float on and eventually flow through a silicon wafer and dried in a vacuum. The wafer with the cellulose whiskers were mounted in a specialized holder and platinum coated for 45 s to minimize the charging effect. The dynamic mode atomic force microscopy (AFM) measurements were performed using a Seiko Instruments SPA400 instrument with an SPI 4000 controller. The cantilever was fabricated from Si_3N_4, and the tip was a model SI-DF40 (spring constant: 42 N/m; resonance frequency: 250–360 kHz). For the AFM analysis of the cellulose whiskers, a droplet of the aqueous whisker suspension was allowed to dry on the surface of a silicon wafer. The tensile properties of the electrospun mats were determined using a universal testing machine (Model no. 4200, Instron, USA) at room temperature with a gauge length of 25 mm and at a crosshead speed of 25.4 mm/min. The property values reported herein represent the average of the results for tests run on at least six specimens. The samples were vacuum-dried for 24 h at room temperature before the measurement.

Results and Discussion

The bacterial cellulose pellicles were cultivated in a flask for 2 weeks using glucose as the sole carbon source. After extracting the cells with a 0.2 M NaOH solution for 48 h, the yellowish cellulose pellicles turned white, as shown in Figure 1(a). The FESEM image of the dried pellicle surface showed a random assembly of microfibrils with a

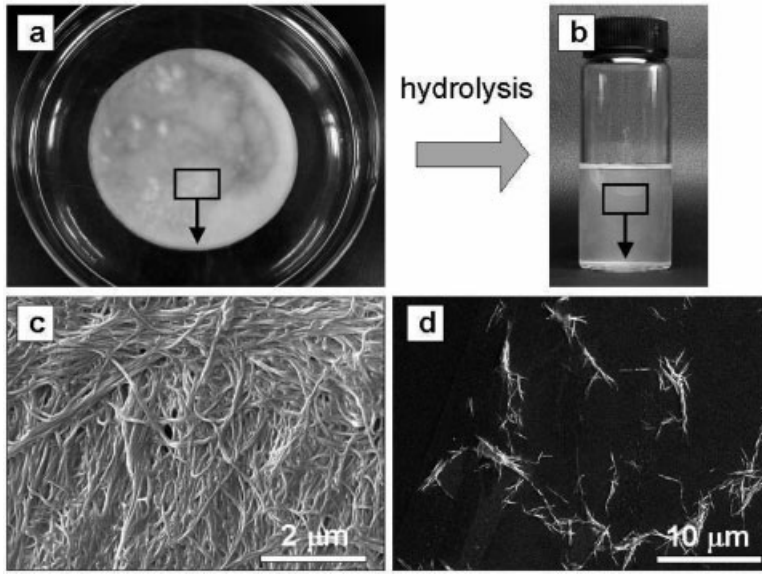

Figure 1.
Preparation of hydrolyzed bacterial cellulose whiskers: (a) Water-swollen gel-like bacterial cellulose pellicle after cell extraction in NaOH solution; (b) Cellulose whiskers dispersed in water; FESEM images of bacterial cellulose (c) before and (d) after hydrolysis.

diameter of approximately 30 nm (Figure 1(c)). The bacterial cellulose was characterized by its ultrafine network structure. The sulfuric acid treated cellulose whiskers formed a stable and well dispersed aqueous suspension without surfactants (Figure 1(b)). The FESEM image of the cellulose fibrils after acid hydrolysis showed that they had a rod like shape, often referred to as whiskers (Figure 1(d)). During the hydrolysis of the bacterial cellulose, sulfate groups were introduced on the surface of the cellulose whiskers. The partially modified whiskers formed a stable suspension in water due to the electrostatic repulsion forces at their surfaces.

The TEM analysis of the dispersion after acid hydrolysis revealed the presence of cellulose whiskers having a needlelike structure (Figure 2). The uranyl acetate staining gave reasonable contrast between the whiskers and the background carbon film on the grid. Although the TEM analysis allowed for the detailed inspection of the individual cellulose whiskers with the aid of the staining to enhance their contrast, the determination of their size was challenging due to agglomeration, but from several images the length was measured to be 420 ± 190 nm and the width 11 ± 4 nm. This correlates with a TEM analysis of cellulose whiskers from wood sources reported elsewhere in the literature.[12]

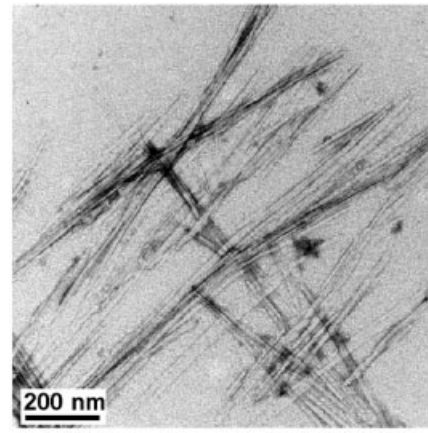

Figure 2.
The TEM image of the cellulose whiskers hydrolyzed from bacterial cellulose fibrils.

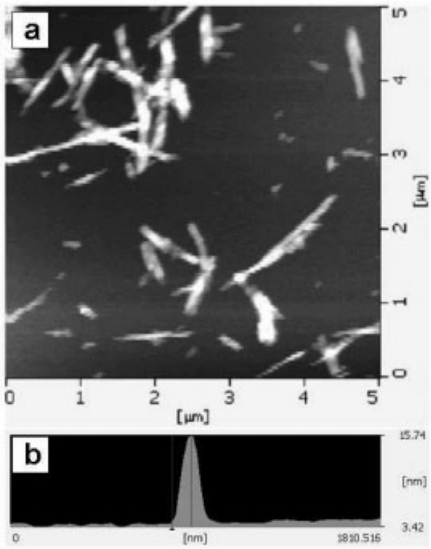

Figure 3.
(a) AFM images of the cellulose whiskers obtained after hydrolysis of the bacterial cellulose fibrils for 16 h and (b) Line scan across one individual whisker.

The AFM analysis of the cellulose whiskers showed this technique to be a good alternative to electron microscopy, without any limitations regarding the contrast and resolution (Figure 3).[12] However, the shape of the whiskers appeared to be different than that observed in the TEM and FESEM images. An estimate of the width of the whiskers could not be obtained due to the broadening effect and possible agglomeration of the whiskers.[12] However, it was possible to estimate the thickness of the whiskers by measuring the difference in height between the silicon wafer surface and the whiskers. Line scans across several individual cellulose whiskers showed a 10 ± 2 nm difference in height between the wafer substrate and the whiskers, as shown in Figure 3. From the TEM and AFM analysis, the width of the whiskers thus appeared to be the same as their thickness.

The addition of PEO to the whisker suspension generated a viscosity and surface tension suitable for electrospinning. The distance between the tip and the collector was 20 cm, and the flow rate of all of the fluids was 0.03 mL/min. As the potential difference between the capillary tip and the counter electrode was gradually increased (from 12 to 24 kV/cm), the drop at the end of the capillary tip elongated from a hemispherical shape into a cone shape. The applied voltages resulted in a jet being initiated near the end of the capillary tip, which induced the accelerated solidification of the fluid jet and the formation of solid fibers with a diameter in the submicrometer range (140 ± 20 nm, $N = 50$). The diameter of the nanofibers was affected by the incorporation of the cellulose whiskers within them (Figure 4). When the content of cellulose whiskers was 0.2 wt% and 0.4 wt%, the fiber diameter was 250 ± 40 nm and 300 ± 40 nm ($N = 50$), respectively. The electrospun nanofibers with different whisker contents showed a relatively increased fiber diameter and broader size distribution as compared with those of the electrospun PEO fiber without whiskers.

The FESEM analysis revealed that the cellulose whiskers were well incorporated into the electrospun fibers (Figure 4). The

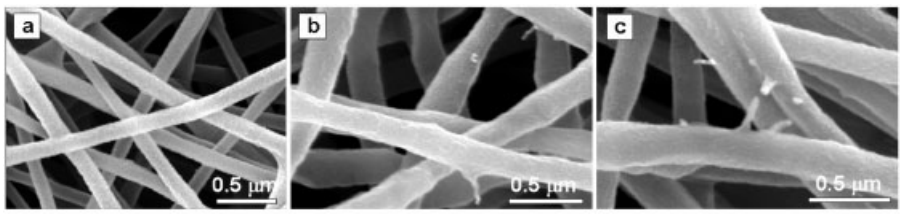

Figure 4.
FESEM image of bacterial cellulose whiskers-incorporated electrospun PEO fiber. (a) 0 wt%, (b) 0.2 wt% and (c) 0.4 wt% of whiskers.

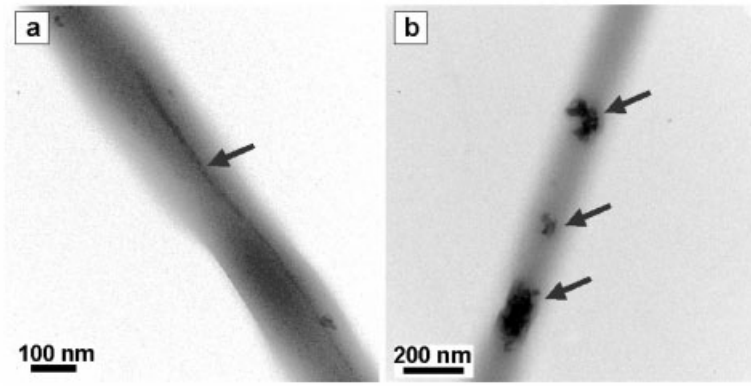

Figure 5.
TEM images of the bacterial cellulose whiskers-imbedded electrospun PEO fibers (0.4 wt%). Cellulose whiskers are (a) aligned and (b) entangled in electrospun PEO fibers.

surface of the electrospun PEO nanofibers without whiskers is very smooth, as shown in Figure 4(a). On the other hand, the addition of the whiskers to the PEO made the fiber surface rough and uneven, while the whiskers were well embedded in the fiber (Figures 4(b) and 4(c)). It is clearly shown that some parts of the whiskers protruded from the side of the nanofibers.

We further investigated the internal structural details of the electrospun PEO nanofibers containing whiskers by TEM. The electrospun nanofibers containing whiskers, shown in Figure 5(a), were well aligned. However, in Figure 5(b), the TEM photograph clearly shows that the embedded whiskers are entangled due to their aggregation during the electrospinning process. Even though the whiskers are considered to be well oriented, partially aggregated whiskers are also observed within the nanofibers.

The values of the tensile modulus, strength and elongation of the electrospun mats are shown in Table 1. After the incorporation of the whiskers (0.4 wt%) into the electrospun PEO fibers, their tensile modulus, tensile strength and elongation were increased by 193.9%, 72.3% and 233.3%, respectively. The existence of the whiskers was effective in improving the mechanical properties of the electrospun mats.

Conclusions

Bacterial cellulose whiskers were prepared by the acid hydrolysis of microcrystalline cellulose. From the TEM and AFM analyses, it was possible to identify individual whiskers, which enabled their sizes and shape to be determined. The cellulose whiskers-reinforced nanofibers were produced by the electrospinning process. The successful formation of electrospun fibers with a diameter of less than 1 μm was observed by FESEM and TEM when using the well-embedded cellulose whiskers. Detailed information was obtained from TEM; the whiskers were well embedded

Table 1.
Mechanical Properties of Electrospun Fibers.

Electrospun fibers	Young's modulus(MPa)	Max. Stress(MPa)	Ext. at Break(%)
PEO	32.7 ± 5.9	1.01 ± 0.15	176.4 ± 44.3
PEO/Cellulose Whiskers (0.2 wt%)	70.4 ± 8.7	1.45 ± 0.16	348.8 ± 80.3
PEO/Cellulose Whiskers (0.4 wt%)	96.1 ± 10.7	1.74 ± 0.09	588.0 ± 102.5

and aligned inside the fibers, even though they were partially aggregated in some of the fibers. It was found that the incorporation of the cellulose whiskers was efficient in enhancing the mechanical properties of the electrospun fibers.

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