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Polycarprolactone Ultrafine Fiber Membrane Fabricated Using a Charge-reduced Electrohydrodynamic Process

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Abstract: This paper introduces a modified electrospinning system for biomedical wound-healing applications. The conventional electrospinning process requires a grounded electrode on which highly charged electrospun ultrafine fibers are deposited. Biomedical wound-healing membranes, however, require a very low charge and a low level of remnant solvent on the electrospun membrane, which the conventional process cannot provide. An electrohydrodynamic process complemented with field-controllable electrodes (an auxiliary electrode and guiding electrodes) and an air blowing system was used to produce a membrane, with a considerably reduced charge and low remnant solvent concentration compared to one fabricated using the conventional method. The membrane had a small average pore size (102 nm) and high porosity (85.1%) for prevention of bacterial contamination. *In vivo* tests on rats showed that these directly electrospun fibrous membranes produced using the modified electrospinning process supported the good healing of skin burns.

Keywords: nanofibers, electrospinning, wound healing.

Introduction

Electrospinning is a simple technique that is widely used to fabricate micro- to nanometer-sized fibers of various polymers. When a sufficiently large electric field is applied to the polymer solution at the end of a syringe nozzle, the solution can be ejected by the coupled effects of the electrostatic repulsion of the surface of the charged droplet and the attraction to a grounded electrode of opposite polarity.[1,2] Since electrospun nanofiber has a high surface-to-volume ratio, it has been used for a number of applications, especially in biomedical fields as a high-performance filtering system, drug delivery system, wound-dressing materials, and for tissue-engineering scaffolds.[3-5] As a wound dressing, the fine pores and high porosity of an electrospun membrane keep exudate from the wound area and inhibit the invasion of exogenous micro-organisms.[6] However, there are still issues regarding the use of electrospun membrane as a wound-dressing material. For example, there is generally insufficient control of the residual charge density and residual solvent in the fabricated membrane. The residual charges can interrupt the stable deposition rate of electrospun fibers, and harmful chemical solvents can affect the wound-healing rate.[7]

This paper describes a modified electrospinning technique that is supplemented with an auxiliary electrode, guiding electrodes, and air blowing system to fabricate micro/nanofibrous membranes. An auxiliary electrode is attached to the nozzle to stabilize the Taylor cone of the polymer solution at the nozzle tip and initial jets, and guiding electrodes are used to reduce the charge on the electrospun fibers and concentrate the electric field in the area between the electrodes. An air-blowing system is used near the nozzle to accelerate the evaporation of the solvent during the process. The amount of charge in the electrospun membrane was characterized to evaluate the stable deposition of electrospun fibers, and the decrease in solvent was measured for different air pressures. We carried out a microorganism protection test in an animal study to demonstrate the feasibility of this method for producing wound dressings.

Experimental

Materials. We used poly(ε-carprolactone) (PCL) (M_n= 80,000; Sigma-Aldrich) and the solvents, *N*,*N*-dimethyl formamide (DMF; Junsei Chemical) and methylene chloride (MC; Junsei Chemical). The electrospinning solution was produced by mixing 80 wt% MC and 20 wt% DMF with 8 wt% PCL. The electrical, surface tension, and rheological

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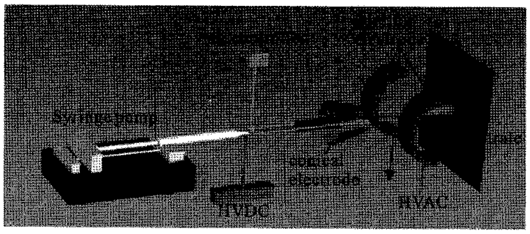

Figure 1. Schematic of a charge-reduced electrospinning system.

properties of the PCL solution used for the electrospinning process were 1.7 μS cm^{-1}, 36 mN m^{-1} and 219 cP, respectively. The PCL solution was placed in a 10-mL syringe fitted with a 22-gauge needle. A syringe pump was used to feed the polymer solution into the needle tip at a fixed flow rate of 7 mL/h.

Electrospinning System. The electrospinning process requires an auxiliary electrode (conical, with an inner diameter near the nozzle of 25 mm), guiding electrodes, and an air-blowing system to fabricate an evenly stacked microfibrous membrane. A schematic of the electrospinning system is shown in Figure 1. The auxiliary electrode was attached to the nozzle to stabilize the Taylor cone at the nozzle tip and the guiding electrodes were curved in a convex manner to concentrate the electric field between the electrodes. The distance between the guiding electrodes and the nozzle was 4 cm. The air-blowing system was attached to the auxiliary electrode and the pressure was controlled by an air-regulator. High-voltage DC electric field strength of 17-24 kV was generated using a high-voltage power supply (SHV300RD-50K, Convertech) between the nozzle tip and the auxiliary electrode. The guiding electrodes produced a high-voltage AC electric field of 2-5 kV/cm at 300 Hz that was generated using a high-voltage amplifier (610E, Trek) and a function generator (AFG310, Tektronix).

The effect of the guiding electrodes on the process was evident in photographs taken by a high-speed camera (FASTCAM Ultima APX RS; Photron). To measure the charges on the membrane, the PCL solution was electrospun onto a 4×4-cm square of polyethylene terephthalate film and transferred immediately into a Faraday cup connected to a nanocoulomb meter (Monroe-284, Monroe). The time between the end of electrospinning and the start of measurement was less than 5 s.

The morphology of electrospun ultrafine fiber membranes deposited on a human hand was observed under a scanning electron microscope (SEM; Sirion). The membranes were sputter coated with gold before the observation. To measure the amount of solvent remaining on the electrospun membranes, the weight of a 5×5-cm square of electrospun membrane was measured using a high-precision balance (BS224S; Sartorius) set in a chamber with constant humidity (45%) and temperature (26 °C). The pore size and porosity of the PCL membranes were measured using an AutoPore IV mercury porosimeter (Micromeritics Instruments, Norcross, GA).

Nanofiber Protection Test. LB plates were used to test protection from microorganisms; these are the plates most commonly used to grow bacteria such as *E. coli.* LB plates are very efficient at stimulating growth and are suitable for many different organisms. Nanofiber was collected on an LB plate for the protection test. *E. coli* (SURE 2) was spread on the nanofiber of the LB plates and the plates were then incubated at 37 °C for 12 h. The electrospun ultrafine fibers were examined using a SEM, and the LB plate was observed using a digital camera.

Sprague Dawley rats (450-500 g) were used for *in vivo* tests to observe the effect of the micro/nanofibrous membranes on wound healing rates. The dorsal hair of each rat was removed with an electric razor. An iron heated to 100 °C was applied for 15 s to a 2×2-cm area on the back of each rat to create the wound. The wound area was then covered with the electrospun nanofibrous membrane, and the burned area of the treated rats was observed using a digital camera.

Results and Discussion

The electrospinning setup consisted of a conical electrode to support stable initial spin jets, guiding electrodes to provide stable spraying of the electrospun fibers on the substrate, and an air-blowing system to accelerate evaporation of the solvent. The initial stability of the Taylor cone is extremely important in obtaining a stable nanofiber membrane. However the initial spun jet can easily become unstable due to processing and environmental conditions.[8-10] An auxiliary electrode was used to overcome these issues. Previous results showed that an auxiliary electrode reduces the instability of the initial jet leaving the apex of the Taylor cone and provides a constant whipping motion to the electrospun fibers. The detailed function and computer-simulated results of the auxiliary electrode are described elsewhere.[8,9] The guiding electrodes are very important in electrospinning material for wound dressings to ensure that the electrospun fibers are deposited stably on the desired target plate. Figure 2 shows high-speed photographs of electrospun ultrafine fibers going past the guiding electrodes. The spun fibers were guided by the AC field window and were concentrated between the guiding electrodes in a stable manner. In Figure 2(a), ultrafine fibers that were electrospun with an applied electric field of 2.65 kV/cm at the nozzle and an auxiliary electrode did not pass through the window of the guiding electrodes when electric field was applied. However, when an electric field of 0.4 kV/cm at 300 Hz was applied to the guiding electrodes, the electrospun fibers easily passed through the gap between the electrodes. The mechanism that concentrates the fibers between the guiding electrodes is very simple. The electrostatic movement of the spun fibers in the window of the guiding electrodes' AC field is subject to a dielectrophoretic force

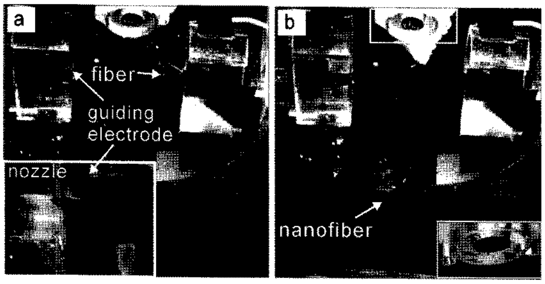

Figure 2. Spraying nanofiber with guiding electrodes: (a) Electrospinning with no electric field on the guiding electrode. (b) Electrospinning with an electric field (0.4 kV/cm and 300 Hz) on the guiding electrode.

(F_D) and a columbic attraction force (F_c).[8,11] Under these electrokinetic forces, the charged fibers converge into the window of the guiding electrode. Several parameters must be controlled in this system to obtain a stable spray of ultrafine fibers. The details of these parameters, such as applied frequency, electric field, dielectric constant, and electrical conductivity, have been described elsewhere.[8,11] Stable deposition of charged electrospun fibers on a target is difficult because of repulsion between the charged fibers. However, the charge on the ultrafine fibers stacked on a target is dramatically reduced by an AC field window generated by guiding electrodes.[12] Figure 3 shows the reduced charge on the electrospun fibers, measured using a nanocoulomb meter connected to a Faraday cup. As shown in Figures 3(a) and (b), the charge on the electrospun PCL membrane decreased dramatically from 5 to 30 s, and the trend of the charge reduction on the membranes was similar. Figure 3(a) shows that the general electrospinning process using a DC field resulted in high charge levels on the fibers, and the amount of charge was proportional to the strength of the applied field. However, using an AC field window generated by guiding electrodes dramatically reduced the amount of charge. As described in our previous work, this phenomenon is a result of the neutralization of the charge on the electrospun fibers as they pass through the AC field window.[12] The AC electric field window applied to the guiding electrodes reduces the positive charge on the fibers generated by the nozzle electrode. Consequently, the fiber mat has a dramatically reduced charge and the electrospun fibers are easily and stably deposited on the substrates without any surface electrical properties.

In addition, this charge reduction process was improved by using an air-blowing system. Figure 3 shows that the amount of charge on the fibers is reduced because the evaporation of the solvent alters the concentration of the solution during the spinning process, which ultimately affects the diameter and charge density of the electrospun fibers.[13]

Removal of the solvent from the electrospun fibers is an important component in achieving stable deposition of the fibers on the target. Figure 4 shows the weight of solvent of electrospun membranes measured using a high-precision balance. PCL solution was electrospun onto a 5×5-cm square and immediately transferred to the high-precision balance. An electrospinning process using an airflow system was

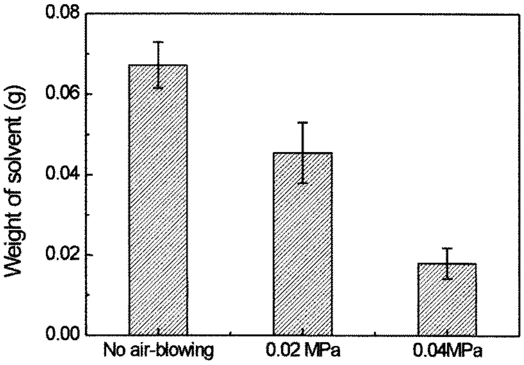

Figure 4. Amount of remaining solvent (DMF, MC) for different air-blowing conditions.

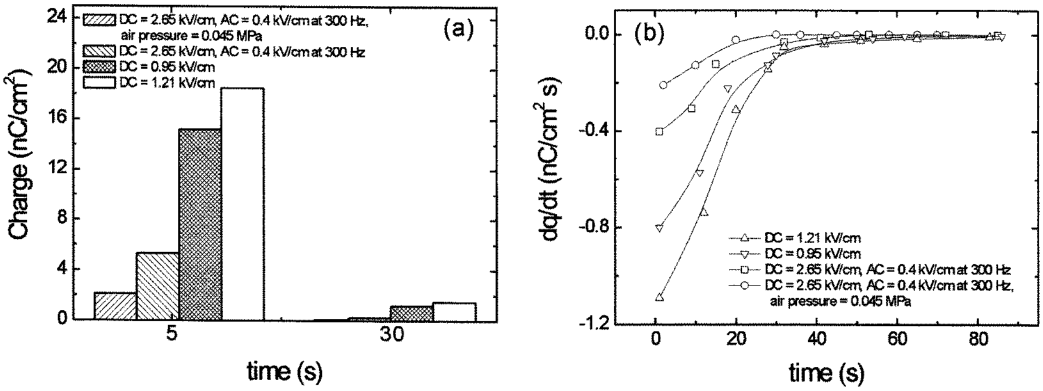

Figure 3. (a) Change in the surface charge on electrospun membranes for 5 and 30 s. (b) Charge gradient for various electric fields.

first suggested by Chu *et al.*,[14] who showed that this increased the solvent evaporation rate and stabilized the formation of ultrafine fibers, and that this process varied with the air-flow rate and the temperature of the airstream. As shown in Figure 4, the conventional electrospun fibers (no-air blowing) held residual solvent in the stacked membrane. However, when the air-blowing system was applied, evaporation of the solvent in the spinning process was accelerated, and the amount of remaining solvent decreased dramatically. The evaporation of the solvent was almost linearly proportional to the air pressure applied.

The charge-reduced electrospinning process is demonstrated in Figure 5(a), which shows a hand coated with a thickness of approximately 10-30 μm in 30 s. The stacked membrane consisted of fibers with diameters ranging from a few nanometers to several micrometers. Figures 5(a) and 5(b) show two different target surfaces (a) a human hand and (b) a hand covered with a latex glove. Each of the targets had different electrical properties. Kessick *et al.* showed that the ability of electrospun carboxy methyl cellulose (CMC) to coat semiconducting and insulating substrates was closely related to the electrical properties of the substrate.[7] However, this difficulty can be resolved by using this technique. As shown in the figure, the ability using this directly electospun technique is not dependent on the electrical properties of the target materials. The AC electric field window applied to the parallel electrodes reduced the charges on the fibers generated by the nozzle electrode, so that the electrospun target has dramatically lowered charges and was independent of the surface electrical property of the target. As shown in the SEM image in Figure 5, the diameters of fibers electrospun without air blowing were in the range 1.47±1.04 μm, whereas those produced with air blowing had diameters in the range 3.2±2.27 μm. The difference in the size of the electrospun fibers may be because the fibers fabricated using air blowing are more highly and randomly elongated in the zone of the whipping area of the electrospinning process.

To investigate the feasibility of this technique to produce wound dressings, we carried out a microorganism protection test. As shown in Figure 6, the ultrafine fibers were electrospun onto an LB plate for the protection test, and the microorganism (*E. coli*, (Figure 6(a)) was spread on the electrospun fiber web using an air spray. After incubation on the electrospun membrane for 12 h at 37 °C, the membrane was removed from the LB plate. Figure 6(b) shows an electrospun membrane deposited on an LB plate for 5 min (flow rate = 10 mL/h) and after drying the membrane, 60 μL of the bacteria were sprayed onto the plate. Figure 6(b) shows that under these conditions, bacteria were still found on the LB plate. However, Figure 6(c) shows that no bacterial colony was observed on the LB plate for the membrane that was electrospun for 15 min at the same flow rate. This result indicates that to protect against the invasion of bacteria and microorganisms the pore size and porosity of the protective membrane must be appropriate. The average pore size and porosity of the membrane was about 102 nm and 85.11%, respectively. According to Huang *et al.*, nanofibrous membranes that acted as an effective shield against the bacteria had a pore size of 500-1,000 nm and a high surface area ranging over 5-100 m^2/g to promote proper fluid management and allow dermal delivery.[15]

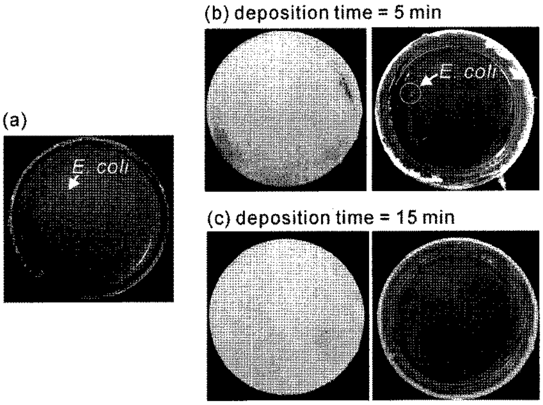

Figure 6. Test of protection provided by nanofiber against *E. coli* (SURE 2): (a) electrospun nanofiber on LB plate; (b) LB plate without nanofiber after bacterial growth.

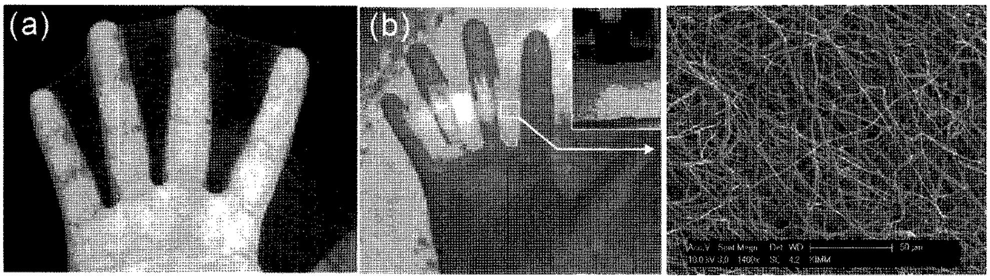

Figure 5. Electrospun ultrafine fibers deposited directly on (a) a human hand and (b) a latex glove. The magnified SEM image shows the electrospun ultrafine fibers on a latex glove.

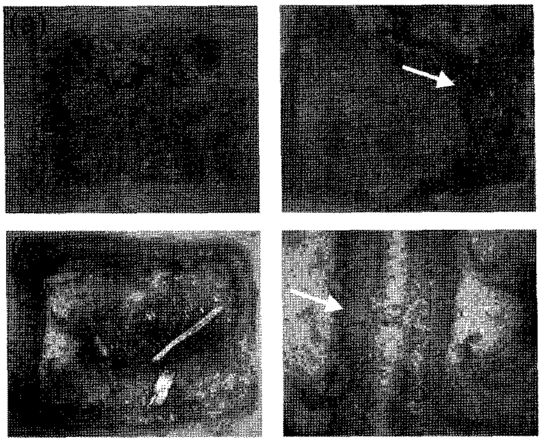

Figure 7. Animal study of wound healing: (a) untreated wound surface; (b) nanofiber applied to wound surface of rat.

The wound surface area in the animal study was created by applying an iron at 100 °C for 15 s to the back of a rat. The burn area was observed after directly electrospinning ultrafine fibers onto it for 15 min. As shown in Figure 7, treatment using nanofiber membranes fabricated by the electrospinning process improved the healing of the skin burn. Figure 7(a) is an image of the untreated burn 7 days after the burn. Skin contractions were observed as well as secondary infections and exudates on the burn wound lesion. Figure 7(b) shows that using ultrafine fiber membrane on the burn area retained more moisture. These results indicate that the electrospinning technique could be used as a new method to fabricate membranes to protect against the invasion of bacteria and microorganisms.

Conclusions

Electrospun ultrafine fiber membranes for wounds were prepared using a modified electrospinning system that was composed of an auxiliary electrode, a guiding electrode, and an air-blowing system. The membrane fabricated using this electrospinning system had considerably reduced charge and low remnant solvent concentration compared to membranes fabricated by the conventional electrospinning process. Bacterial protection and animal tests could estimate that microorganism invasion was inhibited by covering the wound with an electrospun ultrafine fiber membrane.

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