Macromolecular Research, Vol. 22, No. 12, pp 1288-1296 (2014)
DOI 10.1007/s13233-014-2179-6

www.springer.com/13233
pISSN 1598-5032 eISSN 2092-7673

Fabrication and Characterization of Electrospun Curcumin-Loaded Polycaprolactone-Polyethylene Glycol Nanofibers for Enhanced Wound Healing

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Received May 9, 2014; Revised July 30, 2014; Accepted August 10, 2014

Abstract: This study focused on the development of biomedicated electrospun nanofiber mats for preventing wound infections and accelerating wound healing. Polycaprolactone (PCL) nanofibers-loaded curcumin (Cur) and polyethylene glycol (PEG) were generated by an electrospinning technique. The change in surface morphology of the electrospun nanofibers to porous surface after immersion was obtained by field emission scanning electron microscopy (FE-SEM). The biological characteristics of the Cur-loaded PCL-PEG nanofiber mats such as cell viability, cell attachment, anti-inflammatory and antibacterial properties, and *in vivo* wound healing capability were examined. The blending of PEG with PCL resulted in the formation of pores on the nanofibers after immersion, which supports cell viability and proliferation. The mouse myoblast cell line C2C12 showed about 80% viability on the Cur-loaded PCL-PEG nanofiber mat. SEM images showed that the cells could extremely attach and spread out over the surface of the Cur-loaded PCL-PEG nanofiber mat. The inclusion of 0.5 wt% Cur (with respect to PCL) in both the PCL and PCL-PEG blended nanofiber mats inhibited excessive production of nitric oxide (NO) in RAW264.7 mouse macrophages and exhibited good antibacterial activity against *Staphylococcus aureus* (*S. aureus*). *In vivo* wound healing showed that the treatment using Cur-loaded PCL-PEG nanofiber mat significantly increased the rate of wound closure (99%) on day 10 as compared that using PCL nanofiber mat (59%). These results suggest that the PCL nanofiber matrix containing Cur and PEG can facilitate wound healing with cell proliferation and anti-inflammatory properties.

Keywords: polycaprolactone, curcumin, polyethylene glycol, electrospinning, wound dressing.

Introduction

Wound dressing plays a major role to cover the wound for preventing any infection. Throughout history, honey pastes, animal fats, and herbal remedies have been used as wound-healing materials due to their antibacterial properties.[1] In the 20th century, the moist wound care revolution began with the development of polymer synthetics and hybrid polymers, which resulted in better moist-healing conditions. Nowadays, with new biopolymers, antibiotics, and fabrication techniques, more biological approaches are being used to design wound dressings that provide an optimal healing environment, resulting in more rapid healing and less scarring and pain.[1,2] Nevertheless, infection and inflammation caused by bacteria and cells still seriously interfere with the wound healing process. Significant advances are still required for wound dressing in support cell proliferation and less scarring. For this purpose, the use of biopolymers as carriers for delivering necessary amounts of active ingredients such as antibiotics or growth factors to wound sites has been applied to modern wound dressing.[3] Active agents have been loaded in various of form of carriers such as foams,[4] hydrogels,[5] films,[6] sponges,[7] *etc.*, and more recently in the form of polymeric nanofibers.[3,8] These polymeric nanofibers have attracted special attention for use in wound dressings due to their very fine diameter, highly porous structure, and so on.[1,9]

A popular and inexpensive technique for fabricating polymeric nanofibers is electrospinning (ES).[3] Nanofibers fabricated by ES have an extremely large specific surface area, high porosity, and good pore interconnectivity.[10,11] These properties are very similar to the natural extracellular matrix structure that supports cell attachment and proliferation.[12] It was found that active ingredients can be encapsulated directly into nanofibers by electrospinning a mixture solution containing an agent and a polymer.[9,13] Because of their unique properties, the electrospun nanofibers can meet the ideal

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requirements for wound dressing in that they (1) promote a hemostatic phase, (2) provide a moist environment that stimulates wound healing, (3) protect the wound from bacterial penetration, (4) functionalize dressings by incorporating therapeutic agents, and (5) potentially leave no scars.[1,14] The use of biopolymers capable of ES for wound dressing is becoming inevitable because they can generate safe environmental products and easily be washed of the wound surface.[1,3] A variety of biopolymers such as PVA,[3] PLA,[11] poly(urethane),[14] gelatin,[15] chitosan,[16] polycaprolactone (PCL),[17] and some blends of these biopolymers have been electrospun and evaluated for wound dressing. In the present study, we exploited PCL to encapsulate curcumin (Cur), a pharmacological agent, for wound dressing. PCL is a semi-crystalline polymer well known for its nonimmunogenicity, slow biodegradability, and high biocompatibility.[17,18] Due to its non-toxic in nature and flexible mechanical properties, PCL is ideal material for wound dressing and tissue engineering.[18,19] Although PCL nanofiber mat closely mimics the natural extracellular matrix, its hydrophobicity reduces cell attachment. One recently reported technique to improve cell attachment, support cellular proliferation is to fabricate the nanofibers in the form of a porous surface structure. For example, Han *et al.* produced ultrafine porous cellulose triacetate fibers by electrospinning with the high-vapor-pressure solvent methylene chloride (MC).[20] Nguyen *et al.* fabricated core/sheath (PEG/PLA) porous fibers by using a mixture of MC and dimethylacetamide (DMAc).[21] On the other hand, Zhang *et al.*[22] fabricated porous electrospun PCL nanofibers by using of additive gelatin in the solutions followed by their removal in the postelectrospinning process. Pant *et al.* performed simultaneous ES and removal of additive MPEG in order to obtain porous PCLfibers by using a water-bath as a collector.[12]

Curcumin, a polyphenol, is an active principle in turmeric (*Curcuma longa* L.) that imparts the yellowish pigmentation to the plant. It has been widely used to improve wound healing and reducing wound-healing times by increasing fibroblast and vascular density in wounds and quenching free radicals.[23] Many pharmacological activities of Cur have been reported such as antiinflammatory, antiviral, antioxidant,[24,25] wound healing activities.[26] Sidhu reported that Cur enhance wound healing in rats and guinea pigs by reepithelialization of the epidermis and increased migration of various cells, including myofibroblasts, fibroblasts and macrophages.[27] Nevertheless, the use of Cur is limited because of its low solubility in water under acidic or neutral conditions. Many attempts have been made to improve the stability and bioavailability of Cur, such as incorporating Cur in collagen films,[28] encapsulating curcumin in oil-in-water (o/w) nanoemulsions,[29] in biodegradable polymeric micelles,[30] in fibrous chitin sheets,[31] and recently in the form of polymer nanofibers.[32] These studies provide good evidence that Cur incorporated into substrates can maintain its biological activity and enhance wound healing.

In this study, electrospun PCL nanofiber mats containing the therapeutic agent Cur and an additive polyethylene glycol were fabricated with the purpose of preventing wound infections and developing a functional wound dressing. Cur was used to treat inflammation and accelerate wound healing. The high cell affinity and porous surface of the nanofiber mats resulting from the addition of PEG was expected to support cell proliferation. The morphologies of the PCL, Cur-loaded PCL, and Cur-loaded PCL-PEG nanofiber mats were observed using field emission scanning electron microscopy (FE-SEM). The bioactivity of these nanofiber mats was examined *in vitro* with C2C12 cells in terms of the cytotoxicity and the attachment and proliferation. Inflammatory response to the nanofiber mats was assessed through an *in vitro* nitric oxide (NO) assay using RAW264.7 mouse macrophages. The antibacterial activity of the Cur-loaded PCL-PEG nanofiber mats against *Staphylococcus aureus* (*S. aureus*) was evaluated *in vitro* by measuring the optical density of bacterial solutions containing these nanofibers. The *in vivo* wound healing assay using PCL, Cur-loaded PCL, and Cur-loaded PCL-PEG nanofiber mats for treatment were investigated in the mouse model.

Experimental

Materials. PCL (M_n=70,000-90,000) was purchased from Sigma-Aldrich (U.S.A). Curcumin powder was supplied by the Vietnam Institute of Industrial Chemistry (Hanoi, Vietnam). PEG, with an average molecular weight of 1000 DA, was purchased from Alfa Aesar (U.S.A). *N*,*N*-Dimethylformamide (DMF, 99.0%) and dichloromethane (DCM, 99.5%) were purchased from Samchun Chemical Co. (Korea). All the chemicals were used without further purification.

Fabrication of Cur-loaded PCL-PEG Nanofibers. A weighed amount of PCL (8.5 wt%) was dissolved in a mixture of DCM and DMF (at a 90:10 weight ratio) by agitating the mixture with magnetic stirrer for 2 h at room temperature (25 °C). For the preparation of Cur-loaded PCL nanofibers and Cur-loaded PCL-PEG nanofibers, appropriate amounts of Cur (0.5 wt%) and PEG (10 wt%) (with respect to the PCL content) were added and stirred for 1 h. Each of the prepared solutions was introduced into a standard 5 mL plastic syringe attached to a blunt 22-gauge stainless steel hypodermic needle, which was connected to a high-voltage power supply (Chungpa EMI, Korea). A syringe pump (Model KDS200, KD Scientific, U.S.A) was used to control the flow rate of the solution. Electrospun nanofiber mats were collected on a rotating collector covered with an aluminum sheet. All the electrospinning processes were performed at +8.5 kV, a 16 cm needle-tip-to-collector distance, and an 0.8 mL/h solution flow rate. To ensure that DMF did not remain in the nanofiber mats, the mats were dried in a vacuum dryer for 12 h at room temperature.

Morphology and Mechanical Characterizations. The morphology of the electrospun nanofibers was characterized using field emission scanning electron microscopy (FE-SEM) (HITACHI S-4700, Japan). Visualization software (TOMORO

ScopeEye 3.6, U.S.A) was used to determine the average diameter and distribution of the nanofibers from the FE-SEM photographs.

The mechanical performance of the nanofiber mats was characterized using a tensile tester (LR 5K, LLOYD Instrument, UK) with a load cell of 10 N. The length, width, and thickness of the nanofibers were approximately 30 mm, 12 mm, and 70 μm, respectively. The nanofibers were tested with a crosshead speed of 5 mm/min and a preload of 0.1 MPa. All reported tensile strength and tensile stress values represent the average of 6-7 measurements.

***In vitro* Cytotoxic Test.**

Cell Seeding on Cur-Loaded PCL-PEG Nanofiber Mats: PCL, Cur-loaded PCL and Cur-loaded PCL-PEG nanofiber mats (60 μm thick) were cut into circular disks with diameters of either ~7 mm or ~15 mm. These disk specimens were placed at the bottom of the wells of 96-well and 24-well culture plates (Nunc, Denmark), respectively. The plates containing the nanofiber mats were sterilized overnight under UV light. A mouse myoblast cell line (C2C12) was seeded in triplicate onto the nanofiber mats at a density of 1.5×10^4 cells/mL in the 96-well (100 μL) and 24-well (1 mL) culture plates. The cells were then grown at 37 ºC for 1, 3, and 5 days in an incubator.

Cell Counting Kit-8 Assay: The cell viability on the different nanofiber mats was determined from the 96-well plate using a cell-counting kit (CCK-8, Dojindo Molecular Technologies, Japan). After each time interval (1, 3, and 5 days), the culture mediums were removed, and then 10 μL of CCK-8 solution was added to each well of the 96-well plate that contained cells embedded on the nanofiber mats. The plate was incubated at 37 ºC under 5% CO_2 for 2 h. Each assay was carried out in triplicate. The absorbance at 450 nm was measured using a microplate reader (Infinite F50, Switzerland).

Cell attachment to the Nanofiber Mats: Cell attachment was observed in the 24-well plates at each time point using SEM. The seeded nanofiber mats were gently rinsed with phosphate-buffered saline (PBS) and then fixed for 30 min at room temperature using a 2.5 wt% glutaraldehyde (Junsei Chemical Co., Japan) PBS solutions. The nanofiber mats were then dehydrated through a graded series of ethanol solutions (30, 50, 70, and 90% v/v). Samples were dried overnight at room temperature in a clean bench and morphology was examined using SEM (Hitachi S-3600N, Japan).

Statistical Analysis: The statistical analysis of the data was evaluated using one-way analysis of variance (ANOVA) and $p\leq0.05$ was taken as statistically significant. The results are expressed as mean ± standard deviation; the error bars represent the standard deviation.

***In vitro* Anti-Inflammatory Property.** Nitrite production, which is an indicator of nitric oxide synthesis, was determined by Griess reaction assay. Cells of the mouse macrophage cell line RAW264.7 (ATCC) were seeded in triplicate onto sterile PCL, Cur-loaded PCL, and Cur-loaded PCL-PEG nanofiber mats at a density of 4×10^5 cells/mL. Cells were stimulated with 1 μg/mL lipopolysaccharide (LPS) and incubated for 12 h and 24 h. Cells grown on tissue culture plastic without stimulation were used as a control. Supernatants of the cell cultures were mixed with equal volumes of Griess reagent. The optical density at 540 nm was measured and calculated against a sodium nitrite standard curve.

***In vitro* Antibacterial Property.** The optical densities of the bacterial solutions were used to evaluate the antibacterial activity of the Cur-loaded nanofiber mats against the common bacterium *Staphylococus aureus* (Gram positive; ATCC 6538; *S. aureus*).[33] The bacterial suspensions were prepared as follows: 100 μL of bacterial solution containing 10^8 colony-forming units per milliliter (CFU/mL) was diluted to 10^5 (CFU/mL) in Difco™ nutrient broth solution. A 50-mg fragment of each nanofiber mat was introduced into 5 mL of the diluted bacterial solution. To prevent the interference from the color of Cur, each nanofiber mat was incubated separately in Difco™ nutrient broth solution as described above.The mixtures were cultured at 37 ºC in a shaking incubator for 24 h. The turbidity of the medium, which represents the growth of the bacterial cells, was measured using a spectrophotometer (SpectraMax Plus 384, Molecular Devices, U.S.A) at a 600 nm wavelength after a given time (8, 12, 16, 20, and 24 h). The antibacterial efficiency of the Cur-loaded nanofiber mats was then calculated from the following equation:[33]

$$\text{Antibacterial efficiency} = \left(1-\frac{OD_2}{OD_1}\right)\times 100$$

where OD_1 and OD_2 represent the optical densities of the bacteria in the medium and the bacteria in the solutions containing the different nanofiber mats, respectively, for certain incubation times.

Wound Healing Assay. Female BACB/c (14-16 g) were housed under standard conditions with a controlled temperature of 23 ºC and a light/dark cycle of 12/12 h. The animals were anesthetized with Avertin. To create a wound on the animals, the dorsal hairs of the mice were completely shaved and then a certain area of the skin was cut using a circular tracer 8 mm in diameter. The wound areas were sterilized using povidone iodine. Then, the wounds were dressed with the difference nanofiber mats and then covered with commercial gauze. Wounds dressed with only gauze were also prepared as controls. After the necessary predetermined time (5 and 10 days), the reduction in the size of the wound surface was recorded.

Results and Discussion

Morphology of Electrospun Nanofibers. Previous studies have reported that PCL nanofibers have been successfully fabricated by ES using various solvents. In our study, the DCM/DMF was selected as the solvent because DCM is excellent solvent for PCL and Cur while the addition of DMF

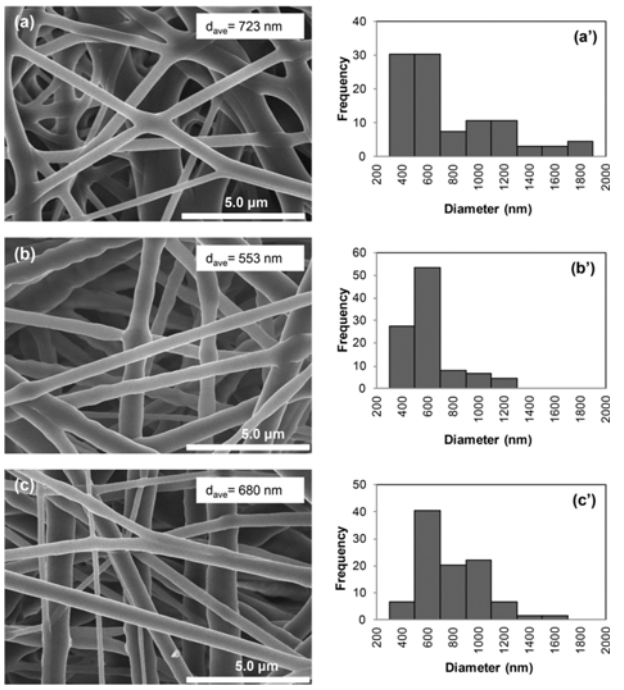

Figure 1. Morphology of (a) electrospun PCL nanofibers, (b) electrospun Cur-loaded PCL nanofibers, and (c) electrospun Cur-loaded PCL-PEG nanofibers. Graphs (a′)-(c′) show the distribution of nanofiber diameters in (a)-(c), respectively.

can improve electrospinability.[34] ES of these solutions was carried out at the fixed electric field of 8.5 kV/16 cm. Morphology of electrospun PCL, Cur-loaded PCL, Cur-loaded PCL-PEG nanofiber mats and their diameter distributions are shown in Figure 1. The incorporation of Cur into the PCL nanofibers not only significantly decreased their average diameter, but also narrowed the diameter distribution of the electrospun nanofibers. The mean diameter of the PCL nanofibers was estimated to be 723 nm with distribution in the range of 300 to 1,800 nm, while that of the Cur-loaded PCL nanofibers was 553 nm with distribution in the range of 300 to 1,200 nm. This is probably due to the change in viscosity of the PCL solution from 425 to 403 cP (Table I). The results obtained in this work are consistent with the results of Zeng *et al*., who reported that the addition of lipophilic drugs (rifampin and paclitaxel) can reduce the diameter size and distribution of hydrophobic electrospun fibers of PLLA.[9,35] Furthermore, it

Table I. Viscosity of PCL Solution and Cur-Containing PCL Solution without and with PEG and the Diameters of the Individual Nanofibers

Polymer Solution	Viscosity (cP)	Fiber Diameters (nm)
PCL	425	723
Cur-Containing PCL	403	553
Cur-Containing PCL with PEG	410	680

is correspond to that the addition of hydrophobic drugs (such as Cur) disturbed the components of polymer solution, lowered the surface tension, and thus enhanced the bending instability during electrospinning.[9] The addition of PEG increased PCL-containning Cur solution viscosity from 403 to 410 cP (Table I). This might be due to interaction between PEG and PCL. Thus, the diameter of the Cur-loaded PCL-PEG nanofibers increase to 680 nm with distribution in the range of 300 to 1,600.

Figure 2 shows the morphology of the electrospun PCL, Cur-loaded PCL and Cur-loaded PCL-PEG nanofiber mats immersed in PBS pH 7 for 1 day and then dried in the vacuum dryer. After immersion, the morphology of the PCL nanofibers showed fused fibers (Figure 2(a)). The diameter of the Cur-loaded PCL nanofibers increased after immersion and the interconnected porous structure was retained. It could be explained that the lipophilic drugs interact with the hydrophobic polymer *via* hydrophobic binding. Cur is the lipophilic drug and highly soluble in PCL/DCM/DMF solution. When the

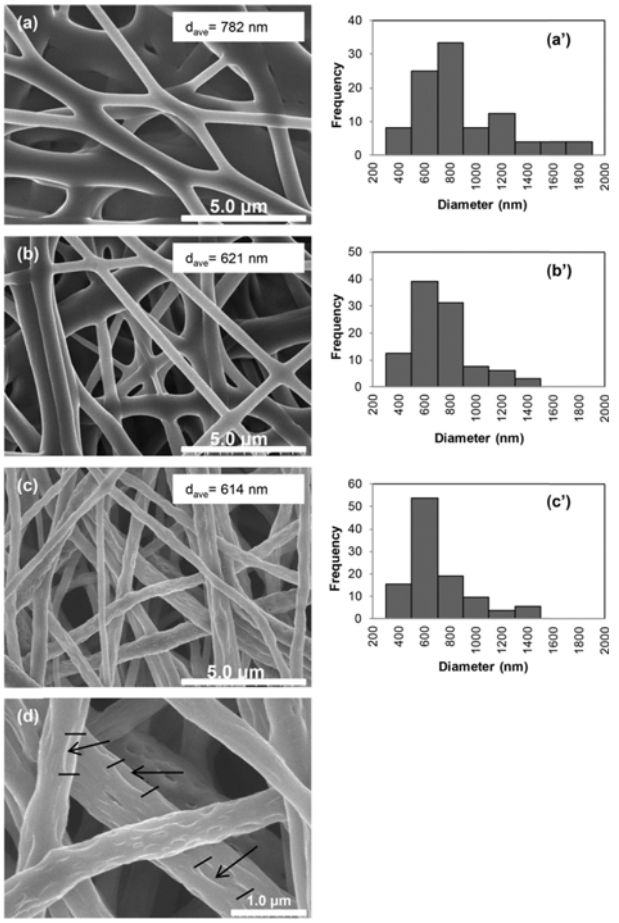

Figure 2. Morphology of (a) electrospun PCL nanofibers, (b) electrospun Cur-loaded PCL nanofibers, (c) electrospun Cur-loaded PCL-PEG nanofibers after immersion in PBS pH 7 for 1 day, and (d) high-magnification image of (c). Graphs (a′)-(c′) show the distribution of the nanofibers diameters in (a)-(c), respectively. The pores are communicate with arrows.

solution jet is rapidly elongated and the solvent evaporated quickly, the Cur remained binding with PCL, and thus acts as trap between polymeric chains.[35] The interconnected porous structure of the electrospun fiber mats could mimic the natural extra cellular matrix, which support for cell attachment and proliferation. With the addition of PEG, the morphology of the Cur-loaded PCL-PEG nanofibers markedly showed a smaller diameter and a narrower diameter distribution with many pores on the surface as compared with the nanofibers before immersion, as shown in Figure 2(c), (c'), and (d), respectively. It is due to the dissolution of PEG in PBS during immersion time. Dissolution of PEG from electrospun PCL nanofibers resulted in the formation of pores on the surface of the nanofibers after immersion.[12] The pores are communicated with arrows. Since bleeding usually occurs during the first stage of wound healing,hemostasis, it is believed that the pores on the surface of the electrospun fibers could be formed in the wound healing process. The pores on the surface of the Cur-loaded PCL-PEG nanofibers could possibly enhance the surface area and assist cell in attachment and spreading. Pant *et al*. fabricated porous PCL nanofibers by water-bath ES using methoxy poly(ethylene glycol) (MPEG).[12] Although their PCL nanofibers had more pores with larger sizes than our resulting nanofibers, they used a large amount of MPEG for fabricating the PCL nanofibers. In this study, Cur-loaded PCL nanofiber mat was prepared with 10 wt% of PEG because PEG dissolution in wounds is limited. In addition, the Cur-loaded PCL nanofiber mat fabricated with 20 wt% of PEG were fabricated and examined after immersion. No significant additional pores were observed than were found for the Cur-loaded PCL nanofibers fabricated with 10 wt% of PEG after immersion.

Mechanical Characterization of the Electrospun Nanofiber Mats. Because the nanofiber mats would be potentially used as wound dressings, they need a certain mechanical strength. Figure 3 shows the results of tensile tests of nonwoven mats of PCL nanofibers, Cur-loaded nanofibers, and Cur-loaded PCL-PEG nanofibers. The PCL nanofiber mat showed mechanical properties, with a tensile strength of 2.5 MPa and elongation at break of 115%. The Cur-loaded PCL nanofibers had a tensile strength of 3.6 MPa and elongation at break of 125%. The tensile strength and strain of Cur-loaded PCL nanofibers increased with the inclusion of Cur, indicating that Cur and PCL showed good compatibility. The nonwoven mat of Cur-loaded PCL-PEG nanofibers had a tensile strength of 3.7 MPa and elongation at break of 167%. The addition of PEG increased the elongation of the Cur-loaded PCL nanofiber mat. It further supports our previous speculation about the interaction between PEG and PCL. These nanofiber mats were found to have higher tensile strength and elongation break than some reported wound dressing.[11,36]

***In vitro* Cytotoxic Assay.** Investigation of the cell viability is important in order to evaluate the cytotoxicity of the nanofiber mats for potential use as a wound dressing. It is well known

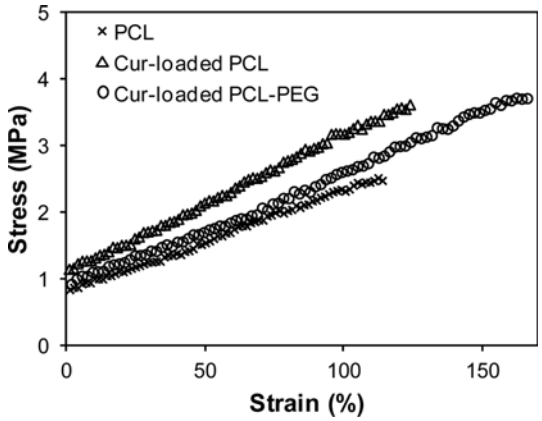

Figure 3. Mechanical strength of PCL nanofibers, Cur-loaded PCL nanofibers, and Cur-loaded PCL-PEG nanofibers.

that the cytotoxic effects of Cur depend on the Cur dose and the type of cell line.[37] In general, the cytotoxic effects of Cur are exhibited at high concentrations of approximately 25 μM.[11,38] In this study, the C2C12 cells were cultured on PCL, Cur-loaded PCL, and Cur-loaded PCL-PEG nanofiber mats for 1, 3, and 5 days. CCK-8 assay was performed to evaluate the viability of the cells corresponding to cell survival, as shown in Figure 4. The cells cultured on polystyrene well-plates as a control gradually proliferated during the 5 days of cell incubation. The viability of the cells seeded on the PCL nanofiber mat was significantly lower than that of cells cultured on the control well. This might be attributed to the hydrophobicity of the PCL nanofibers. For all of the nanofiber mats, there was a negligible difference in cell viability after 1 day culture. However, it is observed that cells significantly proliferated on the Cur-loaded PCL and Cur-loaded PCL-PEG nanofiber mats as compared with the PCL nanofiber mats after 3 days and 5 days. After 5 days culture, more than 70% of the cells were viable on both the Cur-loaded PCL and

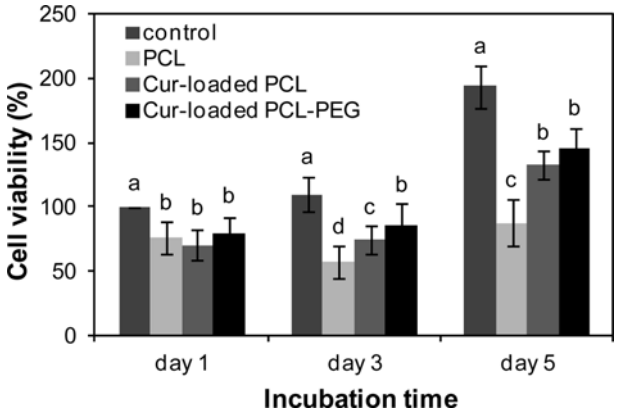

Figure 4. Cell viability assay of C2C12 cells on PCL, Cur-loaded PCL, and Cur-loaded PCL-PEG nanofibers. Values are expressed as mean ± S.D. of three parallel measurements. The cell viability on days 1, 3, and 5 were compared separately ($p \leq 0.05$).

Cur-loaded PCL-PEG nanofibers, indicating their low cytotoxicity, while about 50% of cells were viable on the PCL fibers. The results exhibited that Cur in could induce cell proliferation. In order to test the effect of Cur concentration on cell growth, PCL nanofiber mat and Cur-loaded PCL nanofiber mat without and with PEG (60 μm thick) were cut into circular disk with diameter of 15 mm. The disk specimens were incubated in 1 mL cell culture media. After a given time (1, 3, and 5 days), the extracted Cur was measured using a UV spectrophotometer at 425 nm (UV-1601, Shimadzu, Japan). The results indicated that no significant amount of Cur was observed at those times. It is accepted that Cur concentration in cell culture media is extremely low. The results suggested that the contacting between the cells and the Cur on the surface of the nanofibers affected the cell viability much more than by the concentration of released Cur. Merrell *et al*. showed that on 3% (w/w) Cur-PCL, the viability of HFF-1 cells was more than 70% after 48 h culture.[19] Nguyen *et al*. reported that the inclusion of 0.125 wt% Cur in PLA nanofibers could increase cell proliferation by contact between the cells and Cur on the surface of the nanofibers rather than by the morphology of the nanofibers.[11]

In this study, we added the hydrophilic polymer PEG to form a porous surface on the nanofibers in order to support cell proliferation and attachment. It was found that the viability of cells on Cur-loaded PCL-PEG nanofiber mat was higher than that on Cur-loaded PCL nanofiber mat. The viability of cells seeded on Cur-loaded PCL-PEG nanofiber mat was 78% and 75% after 3 days and 5 days, respectively, as compared with the viability of cells cultured on the control for the same number of days, while the viability of cells on the Cur-loaded PCL nanofiber mat was 67% and 68% after 3 days and 5 days, respectively. The higher cell viability on the Cur-loaded PCL-PEG nanofiber mat might be due to the formation of pores on the surfaces of the nanofibers, which increases cell proliferation and enhances the contact between the cells and Cur on the surfaces of the nanofibers and release of Cur.

Cell Attachment on Nanofiber Mats. Figure 5 shows the SEM images of C2C12 cells on PCL, Cur-loaded PCL, and Cur-loaded PCL-PEG nanofiber mats cultured for 3 days. The figure shows cell attachment and proliferation on the nanofiber mats. Consistent with the results obtained by the CCK-8 assay, the cell attachment on Cur-loaded PCL and Cur-loaded PCL-PEG nanofiber mats was evidently better than that on PCL nanofiber mat. In the cases of the Cur-loaded PCL and Cur-loaded PCL-PEG nanofiber mats, the cells were able to adhere throughout the mats. The results suggest the good biocompatibility of Cur-loaded PCL nanofibers at this Cur percent. The high proliferation of cells on Cur-loaded PCL-PEG nanofiber mat is attributed to the pores on the surface of the Cur-loaded PCL-PEG nanofibers and the presence of small amounts of PEG remain in the fibers. The resulting porous surface nanofibers could provide a suitable platform for the growth of cells in extracellular matrices for better cell attachment and proliferation, consistent with results obtained in a previous study.[12]

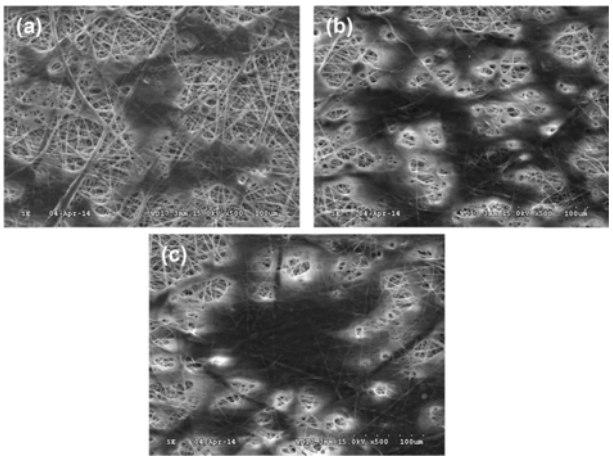

Figure 5. The morphology of C2C12 cells attached to (a) PCL nanofibers, (b) Cur-loaded PCL nanofibers, and (c) Cur-loaded PCL-PEG nanofibers after 3-day culturing.

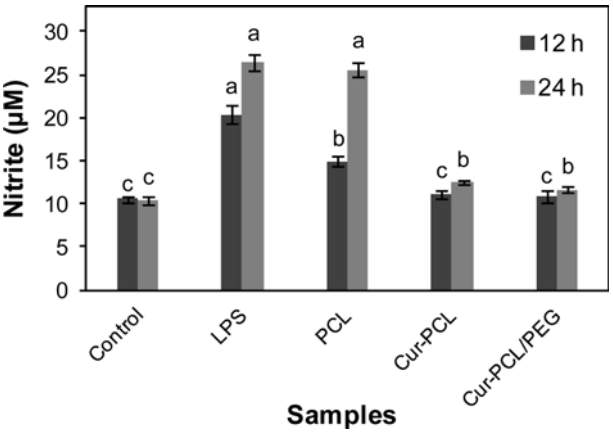

Figure 6. Effect of PCL, Cur-loaded PCL, and Cur-loaded PCL-PEG nanofibers on nitric oxide production in macrophage RAW 264.7 cells. The RAW264.7 cells were incubated with LPS (1 μg/mL) and the nanofibers for 12 h and 24 h. Values are expressed as mean ± S.D. of three parallel measurements. Means with different superscripts are significantly different at $p \leq 0.05$.

***In vitro* Anti-Inflammatory Effect.** Excess production of NO is implicated in the development of multiple organ failure, contributes to tissue damage and enhances inflammatory response.[39] The ability of PCL, Cur-loaded PCL, and Cur-loaded PCL-PEG nanofiber mats to inhibit NO production after stimulating RAW264.7 mouse macrophages with LPS is shown in Figure 6. RAW264.7 showed a significant reduction of NO production when seeded on Cur-loaded PCL and Cur-loaded PCL-PEG nanofiber mats as compared with cells seeded on PCL nanofiber mats. For the cells seeded on PCL nanofiber mat with LPS stimulation, high NO production was observed

(14.98 μM and 25.57 μM after 12 h and 24 h, respectively, $p \leq 0.05$). By contrast, the NO production of the solutions containing cells seeded on Cur-loaded PCl nanofiber mats fabricated without and with PEG, were 11.22 μM and 10.80 μM, respectively, after 12 h and 12.48 μM and 11.69 μM, respectively, after 24 h ($p \leq 0.05$). Since NO production is a major proinflammatory mediator concerned with pathogenic infection by bacteria and viruses, these results suggest that Cur-loaded PCL nanofiber mats without and with PEG might inhibit inflammation.

***In vitro* Antibacterial Activity.** Wound infection caused by bacteria can delay healing. Thus, the antibacterial activity of the Cur-loaded PCL-PEG nanofiber mats was investigated using the bacterium *S. aureus*. Figure 7 shows the optical density (OD) curves plotted as a function of incubation time for the media containing 10^5 CFU/mL of *S. aureus* and the different nanofiber mats. Bacterial propagation can be evaluated from the turbidity of the bacterial solutions since bacterial cells are opaque; that is, the lower the OD of a bacterial solution, the fewer bacteria that propagated in the solution. The growth rate of the bacteria in the solutions was determined by measuring the OD of the solutions incubated for various amounts of time.

As shown in Figure 7, PCL nanofiber mat did not inhibit the growth of *S. aureus*. The OD of the bacterial solution containing PCL nanofiber mat increased with the same rate as that of the medium. By contrast, the ODs of the bacterial solutions in contact with the Cur-loaded PCL and Cur-loaded PCL-PEG nanofiber mats were much lower than that of the medium, exhibiting effective antibacterial activity against *S. aureus*. The Cur-loaded PCL nanofiber mat showed an efficiency of *S. aureus* inhibition of 95% and 52% at 12 h and 24 h, respectively. The Cur-loaded PCL-PEG nanofiber mat showed 99% and 70% efficiency for inhibiting the growth of *S. aureus* after 12 h and 24 h, respectively. It can be assumed that the antibacterial activity of the Cur-loaded nanofibers was resulted from the contacting between the cells and the Cur on the surface of nanofibers and the released Cur in the media during incubation time. The dissolution of PEG from the electrospun PCL nanofibers caused the formation of the pores on the surface nanofibers, leading more release of Cur, the active ingredient for antibacterial activity (data not shown). This study suggests that the benefits gained from Cur may prevent any bacteria from penetrating, therefore effectively avoiding exogenous infections.

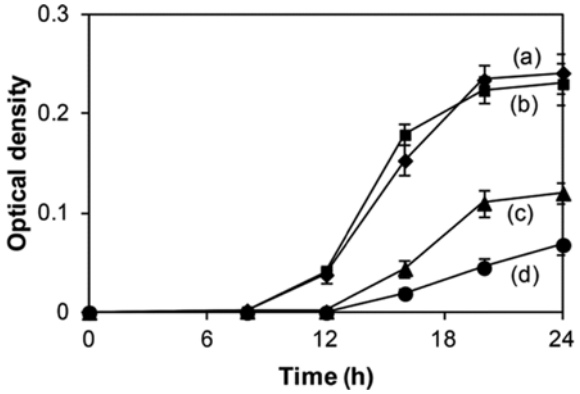

Figure 7. The curves of OD as a function of time for (a) media, (b) PCL nanofibers, (c) Cur-loaded PCL nanofibers, and (d) Cur-loaded PCL-PEG nanofibers. All solutions initially contained approximately 10^5 (CFU/mL) of *S. aureus* before incubation.

***In vivo* Wound Closure Assay.** Studies have revealed that Cur could improve wound healing by increasing fibroblast and vascular density in wounds and prevent oxidative damage.[23] M. Panchatcharam *et al*. reported that Cur treatment significantly increased the collagen, DNA, hexosamine and uronic acid content in the wound.[26] Those components assist the wounds to heal faster without scar formation.[40] D. Gopinath *et al*. reported that incorporation of Cur into a collagen matrix increased wound reduction as compared to collagen film and a control.[28] The incorporation of Cur in a collagen film increased the production of hydroxyproline and protein content, which suggests an increase fibroblasts and collagen in the wound environment as compared to the collagen film and control group.[28] This leads to faster wound healing cascade of inflammation, proliferation, and scar formation. In order to estimate the wound healing effect in the current study, the nonwoven mats of the electrospun fibers were applied to open wounds on the dorsal side of mice. Figures 8 and 9 depict the rate of closure of wounds treated by gauze (control), PCL, Cur-loaded PCL, and Cur-loaded PCL-PEG nanofiber mats. Each wound was observed for a period of 5 and 10 days post-operation. The wound closure rate was calculated as follows:

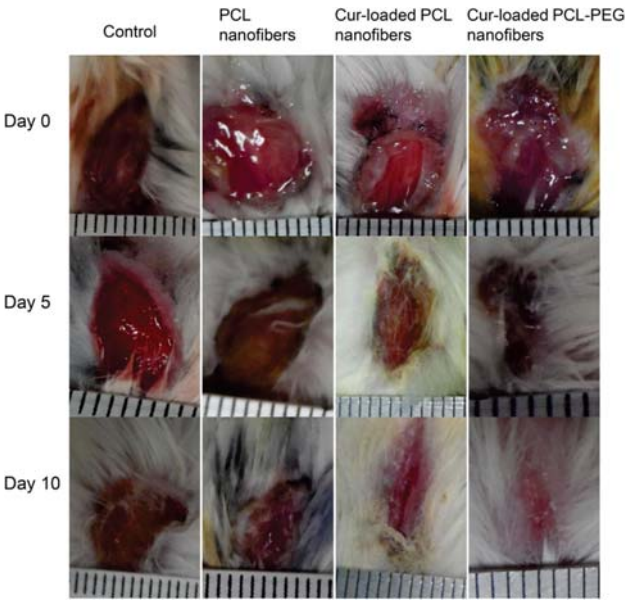

Figure 8. Photographical representation of closure rate of mouse dorsal wound for the control and the nanofiber mats treated on days 0, 5, and 10.

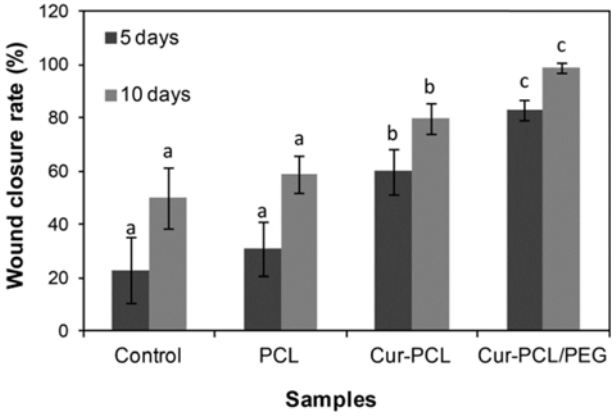

Figure 9. The wound closure rate in control and nanofiber mat-treated wounds after 5 and 10 days. The average values and standard deviations are presented in the graph (n=5). The wound closure rates of different samples at 5 and 10 days were compared separately. The bars with different letters are significantly different $p<0.05$.

$$\text{Wound closure rate (\%)} = \left(\frac{A_0 - A_t}{A_0}\right) \times 100$$

where A_0 and A_t are the wound sizes at day 0 and day t, respectively. There were no significant differences in wound closure rate between the wounds treated with PCL nanofiber mat and the control. On day 10, the closure rate of wounds treated with PCL nanofiber mat and the control were almost 59% and 50%, respectively. After 10 days of the test, the wound closure rate was 90% with the Cur-loaded PCL nanofiber mat. The results demonstrated the ability of Cur-loaded PCL nanofiber mat to enhance the wound cloure rate. J. G. Merrell *et al.* reported that Cur-loaded PCL nanofibers exhibited antioxidant properties and reduced inflammatory induction, which accelerate the rate of wound closure of diabetic mice.[19] The mouse treated with Cur-loaded PCL-PEG nanofiber mat showed more significant wound reduction than the mouse treated with Cur-loaded PCL nanofiber mat. The closure rates of wounds treated with Cur-loaded PCL-PEG nanofiber mat were 80% and 99% after 5 days and 10 days, respectively. On day 10, a new layer of the skin with little scar was formed instead of the wound treated with Cur-loaded PCL-PEG nanofiber mat. These results are consistent with that of the CCK-8 assay and cell attachment test, thus indicating that the addition of PEG into the Cur-loaded PCL nanofibers can improve cell adhesion and support cellular proliferation by forming porous surface structure that supports wound healing.

Conclusions

In this study, continuous nanofibers of PCL and blends of the polymer with Cur and PEG were successfully fabricated by the electrospinning process. For the first time, the combination of therapeutic Cur with antioxidant, antiinflammatory properties and high cell affinity, porous surface resulting from addition of PEG exhibited a useful and convenient method for wound dressing application.The addition of the drug Cur reduced the diameter size and distribution of the electrospun fibers and maintained the interconnected porous structure. The addition of PEG into the Cur-loaded PCL nanofibers led to the formation of many pores on the surfaces of the electrospun nanofibers after immersion in PBS pH 7. From the cell culture and cell attachment results, the porous Cur-loaded PCL-PEG nanofibers were found to have increased biocompatibility. The Cur-loaded PCL-PEG nanofiber mat showed good anti-inflammatory and antibacterial activity toward RAW264.7 mouse macrophages and *Staphylococcus aureus*, respectively. On day 10 of the wound closure assay, a new layer of the skin with little scarring was formed instead of the wound treated with Cur-loaded PCL-PEG nanofiber mat. Therefore, this study suggests the potential use of Cur-loaded PCL-PEG nanofiber mat as an effective wound dressing.

Supporting Information: Information is available regarding the experimental procedure for the detailed morphology characterization and measurement of amount of curcumin released from the PCL nanofibers. The materials are available *via* the Internet at http://www.springer.com/13233.

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