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Enhancement of Diabetic Wound Repair Using Biodegradable Nanofibrous Metformin-Eluting Membranes: in Vitro and in Vivo

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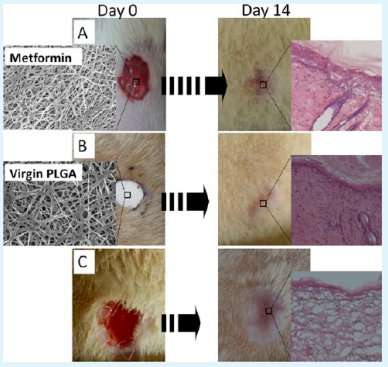
ABSTRACT: This work developed biodegradable nanofibrous drug-eluting membranes that provided sustained release of metformin for repairing wounds associated with diabetes. To prepare the biodegradable membranes, poly-D-L-lactide-glycolide (PLGA) and metformin were first dissolved in 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) and were spun into nanofibrous membranes by electrospinning. An elution method and an HPLC assay were utilized to characterize the in vivo and in vitro release rates of the pharmaceuticals from the membranes. The biodegradable nanofibrous membranes released high concentrations of metformin for more than three weeks. Moreover, nanofibrous metformin-eluting PLGA membranes were more hydrophilic and had a greater water-containing capacity than virgin PLGA fibers. The membranes also improved wound healing and re-epithelialization in diabetic rats relative to the control. The experimental results in this work suggest that nanofibrous metformin-eluting membranes were functionally active in the treatment of diabetic wounds and very effective as accelerators in the early stage of healing of such wounds.



KEYWORDS: *nanofibrous metformin-eluting membranes, electrospinning, release characteristics, diabetic wound repair*

INTRODUCTION

Wounds associated with diabetes and poor healing are among the most important serious complications that can occur during the acute period following skin injury.[1] The nonhealing of a diabetic wound is the most frequent cause of hospitalization associated with such wounds, and may lead to amputation despite improved standards of care. Moreover, nonhealed ulceration affects 15−25% of people who suffer from diabetes at some time in their lives and imposes a major burden on the health of individual patients and on healthcare budgets.[2] Diabetes-related lower extremity amputation results from pre-existing ulceration in 85% of cases.[3] The pathological factors in the treatment of nonhealed diabetic ulcers include the impaired migration and proliferation of keratinocytes and fibroblasts, decreased collagen accumulation, and delayed re-epithelialization in wounds.[4,5]

The clinical need to develop novel methods of treatment to improve the healing of diabetic ulcers is critical. Metformin, a biguanide, is one of the most commonly prescribed antihyperglycemic agents for treating type 2 diabetes.[6] The therapeutic effect of metformin is not limited to its lowering blood glucose by reducing in hepatic glucose output, as revealed by evidence of its pleotropic effects.[7−9] Metformin is also an insulin enhancer and can increase sensitivity to insulin.[10] Additionally, resistance to insulin has been found to impair the healing of excisional cutaneous wounds by delaying the contraction and re-epithelialization thereof.[11] This fact sheds a new light on the use of metformin in the treatment of diabetic wounds.

Poly(lactic-co-glycolic acid) (PLGA) is a biodegradable materials that has been extensively investigated as a therapeutic delivery vehicle because of its biodegradability and biocompatibility. Elsner et al. developed a wound dressing that combines the advantages of occlusive dressings with biodegradability,[12,13] comprising a composite material that is coated with a porous PLGA matrix. It was designed to protect the wound until it is no longer needed, when it dissolves by hydrolytic degradation to form nontoxic end products. Furthermore, the presence of drugs in the PLGA nanofibers increases the wettability of the electrospun matrices and acts as an excellent scaffold, promoting cell proliferation and growth.[14]

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In our earlier work, the present authors developed novel sandwich-structured nanofibrous membranes to deliver drugs sustainably to repair infected wounds.[15] The present work develops biodegradable nanofibrous, metformin-eluting membranes from PLGA by electrospinning for the purpose of dressing diabetic wounds. Topical application of a PLGA scaffold with metformin was hypothesized to support re-epithelialization and augment cutaneous wound closure in a diabetic animal model.

After electrospinning, the morphology of electrospun nanofibers was elucidated by scanning electron microscopy (SEM). The in vitro and in vivo release characteristics of pharmaceuticals from the membranes were studied. The efficacy of the nanofibrous metformin-eluting PLGA dressing in repairing wounds in diabetic rats was studied. Epithelialization and inflammation at the wound site were also histologically investigated.

MATERIALS AND METHOD

Fabrication of Nanofibrous Membranes. The used PLGA was a commercially available material (Resomer RG 503, Boehringer, Germany) with a lactide:glycolide ratio of 50:50 and an intrinsic viscosity of 0.4. Metformin ($C_4H_{11}N_5$) and 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) were purchased from Sigma-Aldrich (Saint Louis, MO, U.S.A.).

Two types of membrane, drug-eluting PLGA membranes and virgin PLGA membranes, were fabricated by the electrospinning technique. The electrospinning setup in this work involved a syringe and needle (with an internal diameter of 0.42 mm), a ground electrode, an aluminum sheet, and a high voltage supply.[16] The needle was connected to the high voltage supply, which generated positive DC voltages and currents up to 35 kV and 4.16 mA, respectively. The materials from which the drug-eluting membrane (PLGA, 240 mg; Metformin, 40 mg) and virgin PLGA membrane (PLGA, 280 mg) were first dissolved in 1 mL of HFIP. A few test trials were first completed to identify the appropriate processing conditions for the electrospinning of nanofibrous membranes. The conditions thus obtained include: The solutions were delivered and electrospun by a syringe pump with a volumetric flow rate of 3.6 mL/h. The distance between the tip of the needle and the ground electrode was 12 cm, and the positive voltage that was applied to polymer solutions was 17 kV. All electrospinning experiments were performed at room temperature. Nanofibrous membranes with a thickness of approximately 0.2 mm were thus obtained. All electrospun membranes were placed in a vacuum oven at 40 °C for 72 h to evaporate the solvents.

SEM Observation. The morphology of electrospun nanofibers was observed on an SEM (Hitachi S-3000N, Tokyo, Japan) after they were coated with gold. The diameter distribution were obtained by analyzing SEM images of 20 randomly selected fibers for each test sample ($n = 3$)[16] using a commercial Image J image software (National Institutes of Health, Bethesda, MD, USA).

The densities of the electrospun nanofibers were calculated by dividing their mass by their volume. The apparent porosity of the nanofibrous membranes was calculated using the following equation.[17]

$$\text{pore}\ (\%) = \left\{1 - \frac{\rho_{\text{membrane}}}{\rho_{\text{polymer}}}\right\} \tag{1}$$

where ρ_{membrane} and ρ_{polymer} denote the densities of the nanofibrous membrane and the polymer, respectively.

Mechanical Properties. The mechanical properties of nanofibrous membranes were evaluated using a Lloyd tensiometer (AMETEK, USA) that was equipped with a 100 N load cell, in a manner consistent with the ASTM D638 standard. A membranous strip that had dimensions of 10 mm by 50 mm and was free of air bubbles or physical imperfections was held between two clamps that were separated by 3 cm. During measurement, the membrane was pulled by the top clamp at a rate of 60 mm/min through a distance of 10 cm before the clamp was returned to its starting point. The force and elongation before the membrane was broken were recorded. Measurements were made five times on each membrane. The tensile strength and elongation at breakage were calculated as follows.

$$\text{tensile strength (MPa)} = \text{breaking force (N)}/\text{cross-sectional area of sample (mm}^2\text{)} \tag{2}$$

$$\text{elongation at breakage (\%)} = (\text{increase in length at breaking point (mm)}/\text{original length (mm)})100\% \tag{3}$$

Contact Angle of Water. The water contact angles of nanofibrous membranes were measured using water contact angle analyzer (First Ten Angstroms, USA). Samples with dimensions of 1 cm × 1 cm were cut from the membranes and placed on the testing plate after distilled water was carefully dropped on their surfaces. The contact angles were measured using a video monitor. The contact angles of both the metformin-eluting and the virgin PLGA membranes were measured.

Water Uptake Capacity. The water uptake capacities of metformin-loaded PLGA and virgin PLGA nanofibers were determined. Electrospun nanofibers were first immersed in distilled water. They were then removed from the water for 0.5, 1, 2, 3, 8, and 24 h and weighed after the surface water was removed using filter paper. The water content (WC, %) was calculated as follows.

$$\text{WC}(\%) = ((W - W_0)/W_0)100 \tag{4}$$

where W_0 and W are the weights of the samples before and after immersion in water for various times, respectively.

Preparation of Diabetic Rats and Wound Healing Test. Eighteen Sprague–Dawley rats that weighed 358 ± 32 g were used in this work. All animal-related procedures received institutional approval, and all of the studied animals were cared for in a manner consistent with the regulations of the National Institute of Health of Taiwan under the supervision of a licensed veterinarian. Before and during the experiments, all rats were housed in individual cages in a central animal care facility, with a 12h light-dark cycle and controlled temperature and humidity, and given free access to standard rodent chow and water. Experimental diabetes was induced in rats by a single intraperitoneal injection 70 mg/kg body weight of sterile streptozotocin (STZ) (Sigma, St Louis, MO, USA) in sodium citrate (0.1 mol/L, pH 4.5) immediately before the rats were to be used. The diabetic state was verified 72 h following STZ injections by measuring blood glucose levels of over 300 mg/dl. One week after the initial administration of STZ, the following studies were performed.

After anesthetization, a sterile template with a diameter of 8.0 mm was placed on each side of the middle of the back of each rat and a full-thickness wound to the deep fascia according to the template, was made by excising the skin. The rats were divided into three groups, each of six rats. The drug-eluting membrane (PLGA/metformin matrix) was applied to the diabetic wound of each rat in group A, while the virgin PLGA nanofibrous membrane (PLGA only) was applied to the diabetic wound of each rat in group B. The rats in group C were treated with a conventional gauze sponge as controls. No dressing replaced and no other topical drug was applied during the healing process. The edge of the wound was traced onto a glass microscope slide and its area was determined by planimetry using Image J image software. The area defined by the trace obtained immediately after wounding was used as the original area (day 0 area). Wound closure was expressed as percentage closure of the original wound and was calculated using the following formula.

$$\%\text{wound closure on day } N = ((\text{area on day 0} - \text{open area on day } N)/\text{area on day 0})100 \tag{5}$$

In all groups, blood was obtained daily from a tail vein and its glucose content was measured using OneTouch strips (LifeScan, Milpitas, CA,

USA). Insulin therapy was administered if the animal lost weight and had "high" glucose readings as obtained using insulin glargine (Sanofi-Aventis, Frankfurt, Germany).

The entire wound, including a five mm margin of unwounded skin, was excised down to the fascia on days three, seven, and 14. The tissue samples were embedded in compound that was maintained at the optimal cutting temperature before undergoing frozen sectioning on a microtome-cryostat.

Intradermis (beneath the dressings) tapping, with 19-gauge needle aspiration, was conducted for the rats in group A to whose wounds were applied drug-eluting membranes on days three, seven, 14, and 21, for in vivo release analysis.

In Vitro and in Vivo Release of Metformin. A high-performance liquid chromatography (HPLC) assay was utilized to determine the characteristics of the release of metformin from the nanofibers. The HPLC analyses were performed using a Hitachi L-2200 Multisolvent Delivery System. An XBridge C_{18} 5 μm, 4.6 × 150 mm HPLC column (Waters) was used to separate the metformin. The mobile phase contained 99.9% acetonitrile (Mallinckrodt, U.S.A.) and phosphate-buffered solution (Sigma-Aldrich, Saint Louis, MO, U.S.A.) (65/35, v/v). The absorbency was monitored at a wavelength of 233 nm and a flow rate of 1.0 mL/min. All experiments were performed in triplicate and the samples were diluted to bring the unknown concentrations into the range of the assay standard curve. A calibration curve was obtained for each set of measurements (correlation coefficient >0.99). The elution product was identified and quantified using the highly sensitive HPLC system.

The characteristics of the release metformin in vitro from the nanofibrous drug-eluting membranes were determined using an elution method. Each of the samples with 8 mm in diameter was cut from the electrospun membranes; it placed in a glass test tube (one sample per test tube, total number = 3) with 1 mL of phosphate buffer solution (0.15 mol/L, pH 7.4). The glass test tubes were incubated at 37 °C for 24 h before the eluent was collected and analyzed. Fresh phosphate buffer solution (1 mL) was then added daily for 30 days. The metformin concentrations of the excised tissue in vivo were also determined by the HPLC assay. All samples ($N = 6$) were assayed after dilution with phosphate-buffered saline and were assessed using the standard assay curve.

Statistics and Data Analysis. All data are presented as mean ± standard deviation. One-way ANOVA was used to compare the data to identify statistically differences. Within ANOVA, the post hoc Bonferroni procedure for making multiple comparisons was utilized to detect significant differences between pairs among groups. Differences were considered statistically significant for $p < 0.05$. The data were analyzed using SPSS software (version 17.0 for Windows; SPSS Inc., Chicago, Illinois, USA).

RESULTS

Electrospinning of Nanofibrous Membranes. In this work, two forms of nanofibrous membrane were successfully fabricated by electrospinning. Electrospun nanofibrous membranes were obtained using suitable process parameters, including solvent, polymer concentration, and flow rate. Figure 1 presents the SEM micrographs of the electrospun nanofibers at a magnification of 5000×. The diameters of the electrospun metformin-eluting PLGA nanofibers (443 ± 121 nm) (Figure 1A) were significant smaller than the virgin PLGA nanofibers (772 ± 326 nm) (Figure 1B) ($p < 0.01$), and the porosity of the nanofibrous membranes was high (84.0 ± 1.1% and 80.5 ± 2.0%, for the metformin-eluting PLGA and virgin PLGA nanofibers, respectively) ($p < 0.05$).

The mechanical properties of the electrospun nanofibrous membranes were measured. The experimental results in Figure 2 suggest that virgin PLGA nanofibers had a greater tensile strength than did the metformin-loaded nanofibers (2.52 ± 0.49 vs 0.99 ± 0.15 MPa, respectively) ($p < 0.01$). The virgin

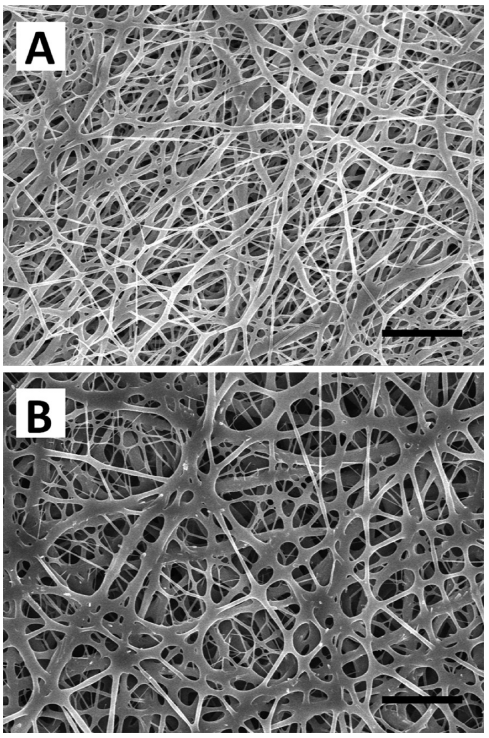

Figure 1. SEM images of two types of electrospun nanofibers; (A) metformin-loaded PLGA, and (B) PLGA only (scale bar: 5 μm). Diameters of the spun PLGA with metformin and PLGA nanofibers ranged were 443 ± 121 and 772 ± 326 nm, respectively. Nanofibrous membranes were highly porous (group A 84.0 ± 1.1%; group B 80.5 ± 2.0%).

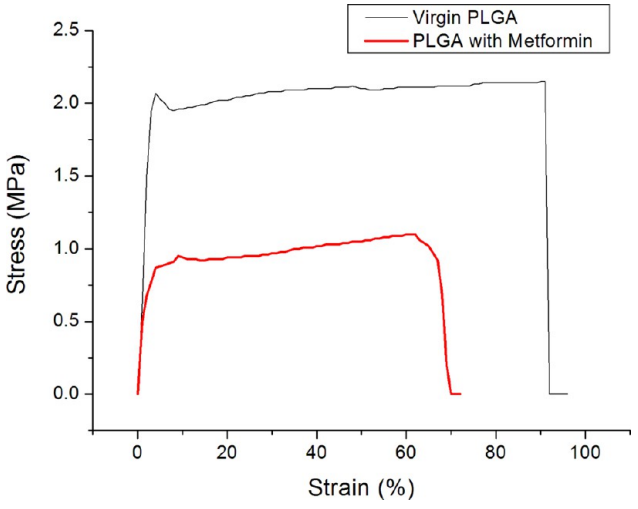

Figure 2. Stress−strain curve of metformin-eluting and virgin PLGA nanofibrous membranes. Top trace is for virgin PLGA with tensile strength of 2.15 MPa and elongation at breakage of 91.9%. Bottom trace is for PLGA with metformin nanomembranes, revealing a tensile strength of 0.87 MPa and elongation at breakage of 67.7%.

nanofibers exhibited greater elongation at breakage (90.0 ± 5.5%) than did the drug-eluting nanofibers (48.2 ± 12.0%) ($p < 0.01$).

Figure 3 shows the measurements of water contact angle on metformin and nonmetformin membranes. The measured contact angles for the drug-eluting and the virgin nanofibers were 53.57 ± 7.76° and 106.33 ± 2.06°, respectively. Clearly,

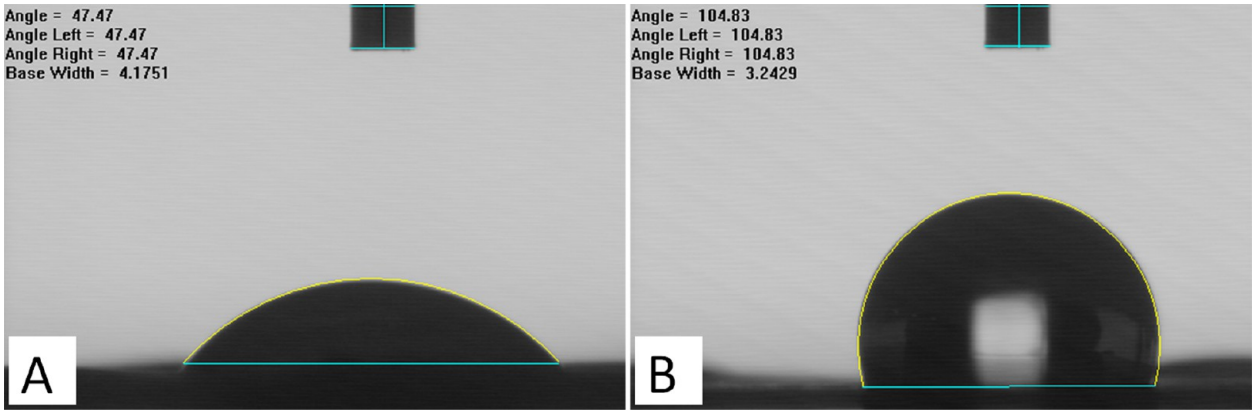

Figure 3. Measured contact angles. (A) Metformin-loaded PLGA, and (B) virgin PLGA nanofibrous membranes. Contact angles were 47.47 and 104.83°, respectively.

adding metformin greatly improved the hydrophilicity of the electrospun nanofibrous membranes ($p < 0.01$).

Variations in the water content of the drug-eluting nanofibers and virgin nanofibers were also studied. The results in Figure 4

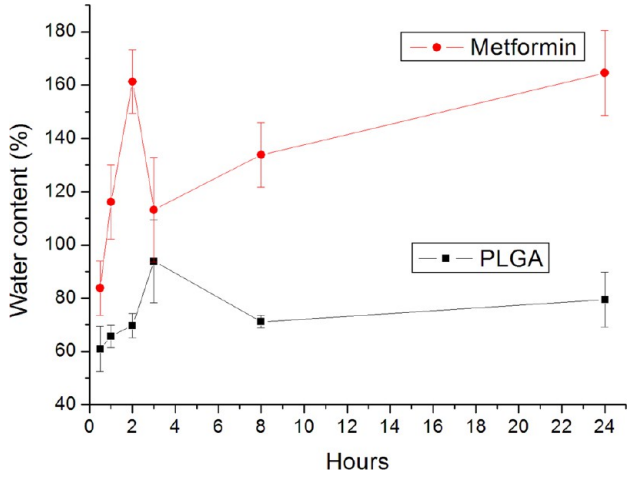

Figure 4. Variation in water content of nanofibers over time. PLGA with metformin reached peak water content (160 ± 12%) after 2 h and PLGA reached peak content (94 ± 16%) after 3 h.

reveal that the water content of the nanofibers increased with time. Whereas the virgin nanofibers reached their peak water content (94 ± 16%) at 3 h, the drug-eluting nanofibers reached their peak water content of 160 ± 12% at 2 h. The capacity of the drug-eluting nanofibers to hold water exceeded that of virgin PLGA nanofibers (ANOVA $p < 0.01$).

In Vitro and in Vivo Release of Pharmaceuticals. Figure 5 plots the daily in vitro release curves of metformin. The drug-eluting membranes continuously released metformin for three weeks, with an initial burst period of 2 days (>200 μg/mL), followed by a second peak of release from days 2−6 (>88 μg/mL) and a third peak of release on days 8−10 (>19 μg/mL), after which the concentration gradually decreased.

In vivo drug levels were also measured for three weeks using the HPLC assay. The drug concentrations revealed that the release peaked on day three (211 μg/mL), after which the cumulative concentration fell progressively.

Wound Healing and Histological Examination. In tests on the healing of diabetic wounds, two full-thickness circular wounds were cut on the back of each rat, parallel to the

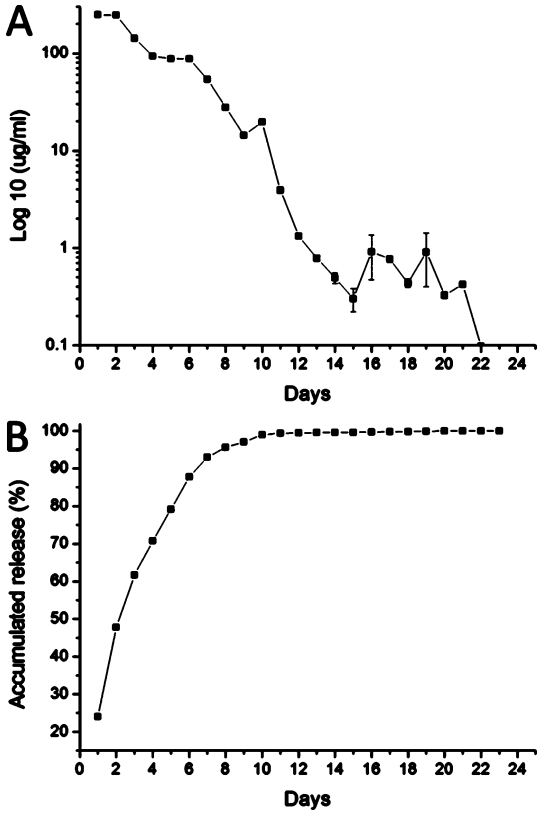

Figure 5. In vitro release of metformin. In vitro (A) daily and (B) accumulated release of metformin from nanofibrous membranes. Release pattern for three weeks includes an initial abrupt burst period of 2 days (>200 μg/mL). A second peak of release rate was observed on days 2−6x (>88 μg/mL), and a third peak of release rate was observed on days 8−10 (>19 μg/mL), after which time concentration gradually decreased.

vertebral column. Figure 6 displays a representative on an animal from each group (metformin-eluting PLGA membrane, virgin PLGA membrane, and gauze sponge groups) on days zero, three, seven, and 14 after treatment. On days three, seven and 14, wound closure in group A was visibly faster than those in groups B and C. On day three and 14, the areas of the wounds in group B and group C were similar. On day seven after surgery, the healing in group B had proceeded visibly faster than that in group C. Figure 7 plots the changes in the areas of the wounds after various periods of healing. Although

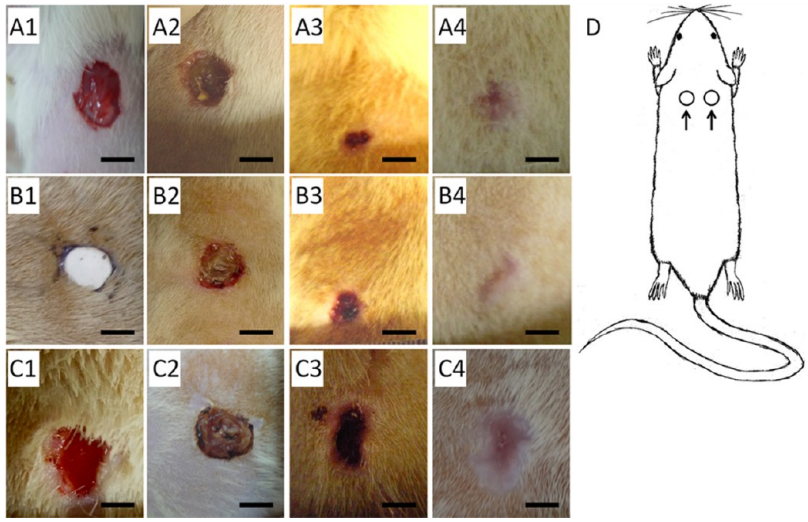

Figure 6. Appearance of healing wound on days 0 (1), 3 (2), 7 (3), and 14 (4) after treatment: (A) PLGA with metformin group and (B) virgin PLGA (C) conventional gauze sponge group (two circular wounds with diameter of 8 mm were prepared on back of each rat, as shown in D) (scale bar = 5 mm). Group A exhibited faster healing than group B and group C (post hoc $p < 0.05$).

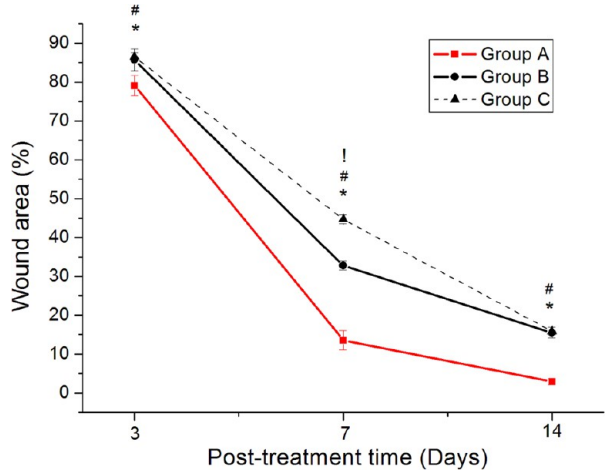

Figure 7. Tests of wound healing in group A (PLGA with metformin nanofibrous matrix), group B (virgin PLGA nanofibrous matrix), and group C (gauge). (* group A versus group B, $p < 0.05$ in post hoc analysis; # group A versus group C, $p < 0.05$ in post hoc analysis; ! group B versus group C, $p < 0.05$ in post hoc analysis). Data are presented as mean ± SD ($N = 4$). At three time points, wound closure in group A had proceeded visibly further than that in group B or group C.

the areas of the wounds of the PLGA nanomembrane and gauze group decreased slowly to 15.5 ± 1.4% and 15.9 ± 1.0% respectively after 14 days, the areas of the wounds covered by the nanofibrous matrix with metformin dropped approximately 2.9 ± 0.8% on day 14. Electrospun metformin-eluting nanofibers (group A) improved the wound healing over that achieved using virgin PLGA nanofibers (group B) or the gauze dressing (group C) (post hoc p all <0.01).

Figure 8 presents images obtained as part of the histological examinations. The nanofibrous scaffolds were well-integrated into the surrounding skin without significant inflammatory response in both PLGA membrane groups. Furthermore, they reveal that nanofibrous metformin-eluting PLGA nanofibers improved wound-healing over that achieved using virgin PLGA naofibers or gauze dressing. Significant infiltration of inflammatory cells was observed in group C on day 3, by comparison with the nanofibrous membranes in groups A and B. On days 3, 7, and 14, a significant variation in re-epithelialization existed among the histological sections of the wounds in the three groups, owing to the migration of keratinocytes from the edges of the wound. At all times, keratinocytes migrated and proliferated fastest in the wounds in group A. Group B exhibited faster re-epithelilization than group C. The nanofibrous groups exhibited less formation of granulated tissue, and less infiltration of inflammatory cells than group C. At two weeks after surgery, the wounds in three groups (Figure 8: A3, B3, and C3) had almost healed, with newly synthesized fibrous tissue and sparse inflammatory cells in the dermis and subcutis, covered by a completely re-epithelialized epidermis in each case. However, the wounds that were treated with drug-eluting and virgin nanofibers exhibited more stratum corneum than those in group C.

DISCUSSION

Biodegradable nanofibrous drug-eluting membranes that sustainably released metformin to repair diabetic wounds were developed. The experimental results herein suggested that the drug-eluting membranes release high concentrations of metformin for more than 3 weeks in vitro and in vivo. The histological images of re-epithelialization at the wound sites revealed that metformin-eluting membranes provide more effective healing of diabetic wounds than virgin nanofibers and gauze dressings. Nanofibrous membranes were functionally active in the treatment of diabetic wounds and were effective as accelerators in the early stages of diabetic wounds healing.

Continuous virgin PLGA nanofibers were obtained at a solution concentration that corresponds to the onset of significant chain entanglements in the polymers. The addition of metformin reduces the percentage of polymer in the solution and the relevant solution viscosity. The mechanical strength decreased accordingly, mainly attributed to the lower percentage of the polymeric matrix that provides the base for strength. In addition, with the same stretching force by the applied electrical field, a solution with a lower viscosity can be more stretched and results in nanofibers with a smaller diameter distribution. On the other hand, compared to

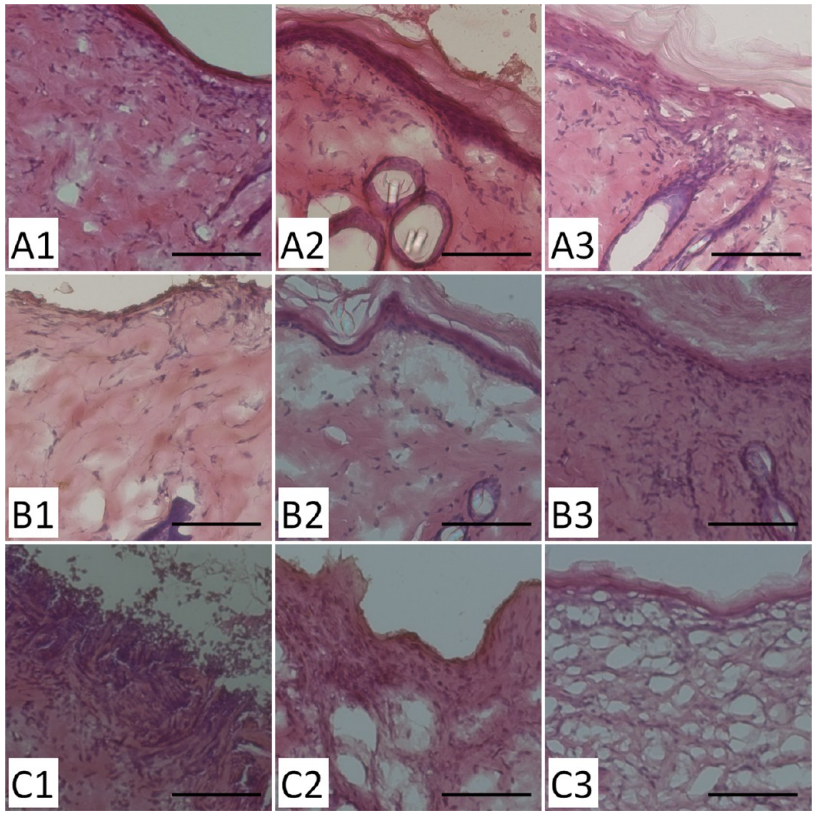

Figure 8. Histological images of (A) PLGA with metformin group and (B) virgin PLGA (C) conventional gauze sponge group, on days 3 (1), 7 (2), and 14 (3) (scale bar = 100 μm). Greater infiltration of inflammatory cells was noted in group C than in other PLGA groups on day three. On days 3, 7, and 14, histological sections of treated wounds in three groups revealed significantly varied degrees of re-epithelialization.

PLGA, metformin is a hydrophilic material.[18] The presence of metformin in electrospun nanofibrous membranes thus increased the hydrophilicity and water uptake capacity of the membranes.

Furthermore, the diameters of the metformin-eluting and virgin PLGA nanofibers developed in this study were comparable with the reported optimum diameter (350−1100 nm).[16] The fabricated nanofibrous matrices thus provided favorable epithelial cell proliferation and accelerated wound healing, mainly due to the fact that metformin in the electrospun matrices promoted re-epithelialization. Tissue engineering is one field in which PLGA has been extensively used as a constituent material of scaffolding systems. Electrospun PLGA fibers have been studied for use in tissue engineering owing to their unique extra-cellular matrix that mimics a nonwoven micro/nanofibrous structure.[19] By adopting the appropriate processing parameters, the fabricated membranes exhibited free of air bubbles or physical imperfections and had a higher porosity than the previously reported mats.[20] As a solvent, HFIP is polar and exhibits strong hydrogen bonding properties enabling it to effectively dissolve a wide range of substances and polymers.[21] Electrospun nanofibrous membranes thus showed good compatibility and interactions between the solvent used (HFIP) and the other ingredients. Therefore, no phase separation in solution or after electrospinning was observed. The nanofibrous meshes showed desirable properties, such as a high aspect ratio, a highly porous structure, and good mechanical strengths,[22] which are favorable for dressing wounds. Additionally, the metformin-loaded nanofibrous PLGA membranes exhibited good extensibility and flexibility, indicating the feasibility of their use in dressings that allow for contraction of the skin during the repair process.

Healing typically proceeds more rapidly under moist conditions than under dry conditions.[23] Conventional wound dressing materials lack any water-retaining property to minimize dehydration. An ideal wound dressing material should control the wetness and humidity of the wound. It should also be permeable to oxygen and carbon dioxide, and therefore promote healing. Accordingly, control of the surface hydrophobicity of PLGA nanofibers to make them better scaffolds with substrates for cell interaction would be desirable. Whereas the virgin PLGA nanofibers were hydrophobic, the metformin-loaded nanofibers were more hydrophilic, mainly because of the presence of metformin. Increasing the surface hydrophilicity of hydrophobic materials reportedly improves cell adherence and, in particular, cell growth. Surfaces with moderate wettability (contact angles of 40−70°) can competitively adsorb cell-adhering proteins, resulting in cell attachment.[24] The porous binding matrix provides sufficient moisture and controlled release of the drugs, preventing the wound bed from becoming dry and thereby promoting healing. The matrix also offers the advantage of eliminating the need for constant wound cleaning and redressing, enabling the body to cope better with healing and reducing the pain and suffering of the patient.[25] Therefore, the presence of metformin in the PLGA nanofibers increases the wettability and hydrophilicity of the electrospun matrices, potentially promoting cell proliferation in the matrices.[16]

On the basis of in vivo and in vitro drug release characteristics, most of the pharmaceuticals were dispersed in the bulk of the PLGA matrix following the electrospinning

process. However, some drug might be located on the surfaces of the nanofibers, causing the initial burst of drug release. After the initial burst, drug release was controlled solely by diffusion and polymer degradation. A relatively steady decline in the rate of release of metformin was therefore observed. Overall, the biodegradable drug-eluting membranes released high concentrations of metformin and were functionally active for more than three weeks in the treatment of diabetic wounds.

Diabetes has been shown to impair immune function and impede wound healing, both of which are critical to survival and recovery from major wound injury.[26,27] Furthermore, hyperglycemia is a risk factor for epithelial downgrowth following skin injury.[28] A previous study demonstrated that metformin promotes peripheral glucose disposal by augmenting insulin sensitivity. Such processes directly counteract metabolic abnormalities that are associated with the hypermetabolic response to injury.[29] Activated insulin receptors are virtually absent in impaired wounds in severely diabetic animals.[30] Therefore, an insulin enhancer could promote the release of vascular endothelial growth factor from keratinocytes in skin wounds.[31] Metformin may change patterns of expression of wound cytokine and chemokine, and reveal some pleiotropic effects, which may improve wound healing rates.[32]

Finally, an improved dressing can provide more rapid healing and a better outcome, with reduced infection, pain, and scarring. It also lowers costs by increasing the rate of healing, reducing the duration of treatment, and allowing for less frequent attention and simpler action by medical professionals. The experimental results herein suggest that the biodegradable PLGA nanofibers that were developed in this study exhibited good biocompatibility and that the delivery of metformin by PLGA nanofibers effectively accelerated wound healing. This finding further reveals that the metformin-eluting PLGA nanofibrous membrane can act as an effective tissue-engineering scaffold for regenerating skin around diabetic wounds.

CONCLUSIONS

This work developed biodegradable nanofibrous drug-eluting membranes that provided the sustained delivery of metformin to repair diabetic wounds. The biodegradable nanofibrous membranes released high concentrations of metformin for more than three weeks. Metformin-eluting PLGA nanofibrous membranes were more hydrophilic and had a greater water-containing capacity than virgin PLGA fibers. The membranes also provided faster wound healing and better re-epithelialization in diabetic rats than did the control materials. The experimental results herein suggest that nanofibrous metformin-eluting membranes were functionally active in the treatment of diabetic wounds and were very effective as accelerators of healing diabetic wounds in the early stages of that process.

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Notes

The authors declare no competing financial interest.

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