Membrane filtration: An unconventional route for fabrication of the flexible and dissolvable, polymer microneedle patches

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Utilization of dissolvable, polymer microneedles (MNs) for transdermal drug delivery offers many advantages such as being painless to patients, biocompatibility, biodegradability, and active and controlled drug release. There are many different approaches for fabrication of such MNs; however, most of them still suffer from tedious procedures, stringent fabrication conditions, expensive equipment, or substantially long processing time. In this work, we applied membrane filtration to fabricate dissolvable, polymer MNs. The polydimethylsiloxane mold having pyramidal wells with through holes was constructed and placed on top a filter membrane. The polymer solution was then dispensed on top of the mold, followed by turning on the vacuum for filtration. It was found that, when using 22% polyvinylpyrrolidone (PVP) solution with molecular weight of 360 000 g/mol, the PVP MNs were obtained within 1 h, which is relatively short time compared to the conventional methods like casting in conjunction with vacuum or centrifugation. Moreover, the MNs as fabricated possessed the similar mechanical strength compared to those by conventional methods and were able to penetrate the rat ear skin with a high insertion ratio. The proposed technique provides an attractive alternative to fabricate dissolvable and flexible, polymer MNs with a simple setup and easy procedures. *Published by AIP Publishing.* [<http://dx.doi.org/10.1063/1.4960206>]

I. INTRODUCTION

The market for transdermal drug delivery (TDD) has been growing significantly in recent years, from approximately US\$25 billion in 2013 to beyond US\$40 billion by 2018 based on a report from Research and Markets. TDD is a self-contained, discrete dosage format that delivers a drug through the skin at a controlled rate into the patient's circulatory system. Compared with other routes of drug delivery such as oral drug administration, TDD offers several merits such as preventing the drug from degrading in the gastrointestinal tract and the first-pass metabolism, sustaining the therapeutic drug level, non-invasive drug application, lower risk of infection, self-administration, and improved patient compliance.^{1–3} The primary barrier to TDD is the stratum corneum layer, which is approximately 20 μm thick, and the viable epidermis layer, which is approximately 300 μm thick, of human skin; these layers provide important protection to the patient from external impurities. Two primary strategies have been used to penetrate the

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skin barrier with TDDs:⁴ one is chemical treatment, which reversibly modifies the physical properties of the skin's lipid bilayer using chemical compounds such as sulfoxides, azones, pyrrolidones, alcohols, and alkanols; the other is physical treatment (e.g., iontophoresis, electroporation, ultrasound technique, photoacoustic methods, and microneedles (MNs)), where reversible pinholes are created to allow the molecules of a drug to penetrate the skin barrier into the patient's circulatory system, thus preventing the risks associated with any additional chemical compounds. Among these techniques used in physical treatment, MNs provide additional benefits such as ease of administration and system integration, being painless and the absence of expensive equipment. MNs have been fabricated with a variety of materials including silicon,^{5,6} metal,^{7,8} photoresist,⁹ and polydimethylsiloxane (PDMS);¹⁰ however, MNs made of these materials are not suitable for direct use due to the risk of residual fragments being left in the human body in the event of broken MNs, the generation of biohazardous sharps waste, or poor mechanical strength. Dissolvable and biodegradable polymer MNs offer certain attractive aspects such as added functionality, active and controlled release, improved resistance to shear-induced breakage, cost effectiveness, sufficient mechanical strength, and additional safety.^{11,12} Although various fabrication techniques have been proposed and demonstrated, such as drawing lithography,⁹ droplet-born air blowing,¹³ electro-drawing,¹⁴ thread forming,¹⁵ low temperature vacuum forming micromolding,¹⁶ particle replication in non-wetting templates (PRINT),¹⁷ photo-polymerization,¹⁸ and dissolving MN on an electrospun pillar array (DEPA),¹⁹ casting in conjunction with vacuum and centrifugation are more widely practiced.^{20–22} For these two methods, prolonged processing time or tedious procedures are typically required to fabricate the MNs with height above 600 μm , which are desirable for effective penetration of skin barrier. Moreover, the footprint of a MN patch is restricted by the diameter of the centrifuge tube. These difficult steps and limitations pose issues to scaling up the manufacturing of these types of MNs.

Therefore, it is clear that dissolvable and biodegradable, polymer MNs cannot be readily fabricated within reasonable processing time using current techniques. The need to develop a relatively fast, simple, reliable, and potentially scalable approach to help commercialize dissolvable and biodegradable, polymer MNs still remains. In this study, we demonstrate a new method to fabricate dissolvable and flexible, polymer MNs via membrane filtration. In addition, we performed mechanical test and skin insertion experiment to show that the polymer MNs as fabricated possess sufficient mechanical strength for transdermal drug delivery.

II. EXPERIMENTAL

A. Mold fabrication

To fabricate the PDMS daughter mold with funnel-like cavities for microfiltration, a glass slide was first cleaned via base washing ($\text{NH}_4\text{OH}:\text{H}_2\text{O}_2:\text{H}_2\text{O} = 1:1:5$) at 75 °C for 1 h. A micro-needle patch (Microchannel Skin System, 3M) with solid, pyramid-shaped microneedles with a 300- μm -by-300- μm square base and a 600- μm height was fixed in a plastic petri dish that was 2" in diameter, which was then brought into contact with the cleaned glass slide. Subsequently, the assembly was gently pressed against each other to ensure contact; PDMS (Sylgard 184, Dow Corning) was then poured into the petri dish, followed by curing in the oven at 65 °C for 1 h and the PDMS daughter mold was obtained.

B. Membrane filtration for the fabrication of polymer microneedles

Figure 1 shows the schematic of fabricating the proposed polymer MNs patch via membrane filtration. The polyvinylpyrrolidone (PVP) aqueous solutions with different concentrations ranging from 10% to 55% (V/V) were prepared. A 20-mg sample of Rhodamine 6G (R6G, Sigma Aldrich) fluorescence dye was dissolved in 40 ml of DI water, and PVP powder (Sigma Aldrich) with a molecular weight of either 40 000 or 360 000 g/mol was dissolved in the prepared R6G solution; the samples were then ultrasonicated for 20 min (ultrasonic cleaner DC200H, Delta). The PDMS daughter mold was placed on top of a mixed cellulose ester

FIG. 1. Schematic of fabrication of polymer MNs via filtration and the optical and scanning electron microscope images of MNs.

(MCE) membrane with different pore sizes ranging from 25 to 1000 nm (VSWP02500, PHWP02500, and HAWP02500 from Millipore; A020A025A and A100A025A from Advantec). The side of PDMS daughter mold with larger openings was in contact with the filter membrane. The assembly was then placed on a Buchner funnel connected to a Buchner flask; the vacuum pump was switched on at a filtration rate of 20 l/min (G-25SA, Ulvac). A sample of 100 μ l of PVP solution with R6G was dispensed on the side of PDMS daughter mold with smaller openings. After a period of filtration time, the residual PVP solution on top of the mold was removed, and the PDMS-MCE patch was baked at 35 $^{\circ}$ C for 10 min. Finally, the PVP MNs patch was obtained by detaching the MCE membrane from the PDMS daughter mold. The time-lapse images of deposition of the PVP inside the funnels at different filtration times were taken using the fluorescent microscope (TE-Ni, Nikon). The microneedles were also examined using scanning electron microscope (SEM) (Hitachi S-3000H or JEOL JSM-6700F).

C. Mechanical test

A universal testing machine (AGS-500NX, Shimadzu Corporation, Kyoto, Japan) was used to conduct the mechanical compression tests.²³ The microneedle array was first placed on the stainless steel base plate, followed by being pressed against a hard metal surface moving downward at a constant speed ($\sim$ 1 mm/s). The force versus the displacement was recorded and the average microneedle force was calculated by dividing the force by the number of needles.

D. *In vitro* skin insertion capability

To evaluate the *in vitro* skin insertion capability of the PVP microneedles produced, Rhodamine 6G-loaded PVP microneedles were inserted into rat ear skin, which had a thickness of approximately 400 μ m, using a constant application force of approximately 14 N for 30 min; the patches were then removed after 30 min. The dissolution of the microneedles and the red spots on the skin were observed and imaged via a stereomicroscope (Leica, Nikon P6000). The insertion ratio for each test sample was calculated by dividing the number of red spots on the skin after insertion by the number of microneedles in the arrays.

E. *In vitro* penetration depth

To determine the skin penetration depth and the molecular delivery in the vertical direction after 30 min of insertion, the penetration of Rhodamine 6G into the rat ear skin at varying depths was viewed via a confocal laser scanning microscopy (LSM 510 META, Zesis, Germany) at an excitation wavelength of 561 nm. Scanning was conducted once at intervals of 10 μm from the skin surface through the z-axis perpendicular to the xy-plane.

III. RESULTS AND DISCUSSION

The top view of the PDMS daughter mold with funnels is shown in Figure 1. It can be seen that the mold has uniform through-holes with approximately 40 μm in diameter at one end and 300 $\mu\text{m} \times 300 \mu\text{m}$ at the other end. After filtration process, the PVP MNs patch with the porous membrane as the MN base was obtained (Figure 1). It was found that not only excellent replication accuracy was achieved but also the MNs patch is flexible owing to the thin porous membrane. Another advantage of using porous membrane is that it reduces the amount of the dissolvable polymer used. Furthermore, if the drug is pre-mixed with the polymer solution to form MNs, using porous membrane could potentially prevent MN base from containing drug, which will not be able to be delivered into human body. Note that formation of MNs without baking at 35 °C can still be achieved in 30-min evaporation at the ambient condition (supplementary material, Figure S1). Moreover, the polymer MNs do not detach from the patch when bending. Since the polymer can be deemed as soft particles, it is hypothesized that minute amount of polymer was retained inside and near the membrane surface, while most of polymer deposited on top of the filter membrane to form the MNs, i.e., blocking filtration²⁴ in conjunction with cake filtration. Retention of the polymer near the membrane surface could provide the MNs with anchorage to the patch. Other water soluble materials like polyvinyl alcohol (PVA) and bovine serum albumin (BSA) were used to form MNs patch via membrane filtration (supplementary material, Figure S2). Compared to the conventional, widely practiced approaches, i.e., casting in conjunction with vacuum and centrifugation, the membrane filtration technique is relatively fast, simple, and reliable. For the casting method, the polymer solution is dispensed on the mold, followed by applying vacuum for few seconds and then turning off. This is required to allow the polymer solution to pre-fill the mold cavity. It is an easy approach; however, foaming may occur during vacuum and some cavities may not be completely filled with polymer solution. Moreover, the evaporation time can range from several hours to at least one day depending on the thickness of the MNs patch. The thicker the MNs patch, the longer the evaporation time. For thinner MNs patch, it took approximately 2 h to form MNs (supplementary material, Figure S3). Note that the MNs were not completely dry as bending of MNs was observed when separating from the mold and the patch was not flat. As to the centrifugation method, two to three cycles of procedures (one cycle refers to the following steps in order: dispensing polymer solution, centrifugation and removing the polymer deposit outside the mold cavity) are required to fill the mold cavity with polymer to form MNs.²¹ In contrast, there is only one step required in the membrane filtration method to form the MNs.

For the polymer to be deposited inside the funnels and form the MNs, selection of the filter membrane with a proper pore size, the molecular weight of polymer, concentration of polymer solution, and filtration time is crucial. It was found that when the average pore size was approximately 1 μm , the PVP with a molecular weight of 360 000 g/mol passed through the filter membrane with some retention and no MNs were formed. As the average pore size decreased to be equal to or less than 450 nm, formation of MNs was observed. For PVP with a molecular weight of 40 000 g/mol, the average pore size needed to be further decreased to be equal to or less than 300 nm in order for formation of MNs. Figure 2 shows the processing window for fabrication of polymer MN with two different molecular weights by using filter membrane with average pore size of 200 nm. It can be seen that, for PVP with a molecular weight of 360 000 g/mol, MNs could not be formed in 30 min when using 22 wt. % polymer concentration. Instead, 50-min filtration time was required in order to obtain MNs. When using 13 wt. % polymer concentration,

FIG. 2. The processing window for fabrication of polymer MNs via filtration. The symbols of circle and cross refer to formation of complete MNs and incomplete or no MNs obtained, respectively. The symbols in dark color and light color refer to polymers with MW 40 000 and 360 000 used, respectively.

the filtration time needs to be further increased up to 90 min. For PVP with a molecular weight of 40 000 g/mol, the polymer concentration needed to be increased up to 45 wt. % in order for formation of MNs within 50 min filtration time. Note that further increasing the concentration of polymer solution led to substantial increase in solution viscosity such that either dispensing the polymer solution on the PDMS daughter mold was difficult or filtration became impractical due to prolonged filtration time.

Figure 3 shows the time-lapse images of the side view of the deposited polymer inside the funnels at different filtration times. It was found that complete polymer MNs could not be obtained within 1 h at lower solution concentrations (e.g., 13 wt. % PVP solution), as shown in Figure 3(a). When using a 22 wt. % solution concentration, the polymer first deposited on top of the membrane and then gradually formed MNs, as shown in the time-lapse images (see Figure 3(b)). A small amount of polymer was deposited at the inner wall of the funnels during the microfiltration process. Since filtration process is operated under constant pressure, the general equation can be written as follows:^{25,26}

$$\frac{t}{V} = \frac{\alpha\mu C}{A^2\Delta P}V + \frac{\mu R_m}{A\Delta P}, \quad (1)$$

where t , V , α , μ , C , A , ΔP , and R_m represent the filtration time (s), the volume of filtrate (m^3), the specific cake resistance (m/kg), the filtrate viscosity (kg/m s), the solid mass per volume of the liquid in the solution (kg/m^3), the filtration area (m^2), the total pressure ($\text{kg}/\text{m}^2 \text{s}^2$), and the initial filter resistance (m^{-1}), respectively. Because the volume of the filtrate, V , is proportional to the mass of the deposited polymer (or the cake), w (i.e., $W = CV$), V can be replaced with w , and, after rearrangement, the above equation becomes

$$A \frac{t}{w} = \frac{\alpha\mu}{\Delta P C A} \frac{w}{C} + \frac{\mu R_m}{C \Delta P}. \quad (2)$$

Note that, since the filtration area is constantly changing during the formation of polymer MNs, an average filtration area is used, which is defined as the cross-sectional area of a cuboid with its height and volume equal to those of the deposited polymer. Also, when the vacuum was turned off, a residual amount of polymer solution remained inside the funnels after the excessive polymer solution on the daughter mold was removed. Therefore, the true amount of the deposited polymer resulted from the filtration process was determined by subtracting the amount of sedimentation during the heating stage. A linear relationship was then obtained by

FIG. 3. (a) Effect of the concentration of the polymer solution on the formation of the polymer microneedles. (b) Time-lapse fluorescence images of the deposited PVP inside the funnels. (c) Relationship between A^*t/w and w/A .

plotting A^*t/w vs. w/A , as shown in Figure 3(c), which indicates that the process of MN formation follows the filtration mechanism.

According to casting in conjunction with vacuum or centrifugation method, it would have been intuitive to place the PDMS mold with the smaller openings in contact with the membrane, while the polymer solution was dispensed at the side of the mold with larger openings. It was found that, instead of forming solid polymer MNs, partially hollow polymer MNs were obtained and the polymer MNs with broken tips were also observed (see Figure S4 of [supplementary material](#)). This could be the result of breakage of the thin, deposited, polymer layer at the tip of the polymer MNs after being released from the mold. It is speculated that formation of the partially hollow polymer MNs could be caused by the occurrence of vortices in the conical converging flow.²⁷ The shear rate of the conical converging flow at the small opening of the funnel could reach approximately 10^5 s^{-1} when the pressure drop is 10^5 Pa , and the solution viscosity is approximately 1 Pa s ²⁸ (see Figure S5 of [supplementary material](#)). At such a high

FIG. 4. Comparison of mechanical strength of MNs made by casting method and membrane filtration.

shear rate, the secondary motion occurs, and the vortex appears as “a barrel of swirling motion, which dances around and across the opening” or as a cyclic motion where the vortex appears, dies out, and reappears.²⁹ This vortex recirculates the polymer upstream and might disturb the formation of solid polymer MNs. Therefore, in order to produce solid polymer MNs, the PDMS

FIG. 5. (a) Rhodamine 6G-loaded PVP microneedles and the insertion of the microneedles into rat ear skin after 30 min of dissolution. (b) Confocal images of the penetration of Rhodamine 6G on rat ear skin at varying depths after insertion of the PVP microneedles for 30 min.

daughter mold with the larger openings needs to be placed in contact with the membrane, while the polymer solution is dispensed at the other side of mold with the smaller openings.

Figure 4 shows the mechanical strength of the MNs fabricated by membrane filtration. As the MNs were compressed, the force increased monotonically, which is similar to what have been observed in the literature. The mechanical strength of MNs fabricated by casting method was also measured for comparison. It can be seen that two curves of the force vs. displacement were nearly overlapped with each other. This indicates that MNs fabricated by these two methods have the similar mechanical strength and deformation behavior, which would suggest that the MNs fabricated by membrane filtration can be used just like those fabricated by other conventional methods.

To evaluate the skin insertion capability and determine the insertion depth of PVP MNs patch, Rhodamine 6G-loaded PVP MNs were inserted into rat ear skin for 30 min. As shown in Figure 5(a), the skin surface exhibited an array of red spots and could not be washed away, indicating that the Rhodamine 6G-loaded PVP MNs were embedded inside the rat skin. A high insertion ratio (i.e., more than 98%) of the PVP MNs was also observed. The penetration depth of the Rhodamine 6G can be estimated using a confocal microscope, and it was found that from the images in the vertical direction, the maximum delivery depth of the Rhodamine 6G was approximately 140 μm (see Figure 5(b)). This result suggests that the PVP MNs fabricated via membrane filtration can puncture the stratum cornea to achieve efficient transdermal delivery.

IV. CONCLUSION

In summary, we have demonstrated that membrane filtration can be applied to fabricate flexible and dissolvable, polymer MNs patch within approximately 1 h. The mechanism of forming the MNs by membrane filtration follows the cake filtration. The polymer MNs as fabricated exhibit sufficient mechanical strength and show a high insertion ratio. The proposed fabrication technique is simple and reliable, and therefore offers a promising alternative to the mass manufacturing of polymer MNs.

SUPPLEMENTARY MATERIAL

See [supplementary materials](#) for PVP MNs fabricated by filtration and dried by evaporation at the ambient condition, PVA and BSA MNs fabricated by filtration, PVP MNs fabricated by casting method, PVP MNs fabricated by filtration where the smaller openings of the mold was in contact with the membrane, and the viscosity measurement of PVP solution with different concentrations.

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