A RAPID MICRO-MOLDING PROCESS FOR FABRICATING POLYMERIC BIODEGRADABLE 3D STRUCTURES USING HYDROPHOBIC ELASTOMERIC MOLDS

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ABSTRACT

We have developed an aqueous-solvent-based micro-molding technique for creating polymeric, bio-degradable micro-structures which can be loaded with delicate bio-active therapeutic agents. The process enables the incorporation of emerging protein- or DNA-based vaccines into sophisticated micro structures, *e.g.*, microneedles, without damaging their biological efficacy as conventional (UV- or temperature-based) micro-molding processes would. As a demonstration, we successfully fabricated gelatin microneedles loaded with a DNA complex using a polydimethylsiloxane (PDMS) mold. The process is proven to be capable of molding microneedles with sufficient sharpness (down to 17 µm radius of curvature at the tips) to penetrate porcine skin and exhibit transfection of DNA complexes.

INTRODUCTION

Fabrication of microstructures for biomedical applications (e.g., microneedles) traditionally entails the use of silicon-based micro/nano fabrication processes due to the required resolution [1], [2]. Such processes, however, are often expensive due to their requirement for cleanroom infrastructure. In addition, the inherent nature of photolithography limits designed structures to simple geometries, typically symmetrical about the normal axis of the substrate [3]. 3D printing is an emerging technology capable of creating more complex non-planar structures; however, this process is typically not used for microfabrication, since commercial bench top 3D printing systems cannot reproduce microscale designs.

Recently, we reported on a polymer shrinking method for miniaturizing 3D printed molds from mm- to µm-scale, resulting in a polydimethylsiloxane (PDMS) mold with complicated micro-features [4]. The use of an elastic mold has the additional advantage of facilitating the release of cast microstructures, especially when slanted or complex micro-features are embodied. This process was used for fabricating biodegradable microneedle arrays. Although capable of reproducing complex 3D structures, this technique, like other conventional micro-molding methods (*e.g.*, thermal injection molding or solvent-casting), presents challenges when casting complex geometries or microstructures with high aspect ratios. This being mainly due to the high viscosity of melted polymer or dissolved polymer solution [5]. For example, premature cooling or gelation of biodegradable polymers prevents a complete filling of micro-cavities in the molds [6], especially when the mold is made of hydrophobic elastomeric material with a large contact angle, such as PDMS. Another limitation is related to the incorporation of emerging protein- or DNA-based molecules into such micro-structures. Most biologics are highly sensitive to elevated temperatures and UV light, both required in many traditional approaches [7].

As a solution to these challenges, we present a modified room-temperature aqueous-solvent-casting method with two critical steps (a controlled degassing followed by dehydration under constant temperature) that enables the fabrication of gelatin microneedles using a pre-fabricated PDMS mold and their incorporation with DNA complexes.

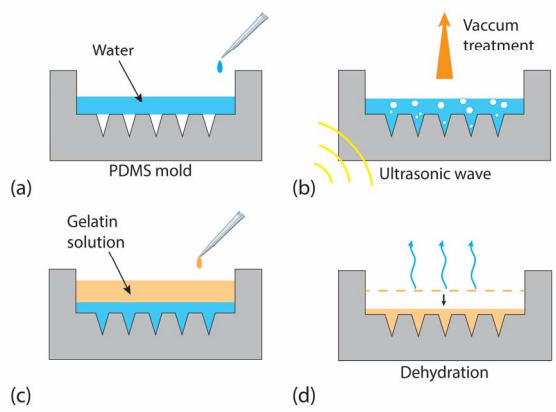

Figure 1: Micro-molding process for gelation casting in a PDMS mold: (a-b) pre-filling micro-patterned mold with water followed by controlled sonication and vacuum degassing at constant temperature; (c-d) depositing and drying gelatin solution; repeat (c-d) for thicker gelatin layers.

FABRICATION

Microneedle fabrication

Figure 1 illustrates the fabrication process involving a controlled degassing and drying process for molding biodegradable microneedles. To start with, a PDMS mold of slanted microneedles is designed and prepared using rapid 3D-print-and-shrink process described in [4]. Briefly, hydrogel is cast around a mm-scale 3D printed master mold which is prepared by a stereo-lithography 3D printing system (Objet Eden350, Stratasys, Ltd., Edina, MN, USA). Subsequent dehydration isotropically shrink the gel. The shrinking steps are repeated iteratively to obtain a PDMS microneedle mold replica (250 µL in volume) containing an array of microneedles.

After preparing the PDMS mold, since surface tension and viscosity of gelatin solution prevent it from reaching the tips of the needles (gelatin also easily solidifies at room temperature before degassing), the micro-cavities are firstly filled with water for the removal of any remaining gas in the mold. Water is selected due to its low viscosity and mixability with gelatin-water solution. Gelatin solution will be added later for mixing with degassed water. 10 µL of molecular biology grade water (Sigma Aldrich) is deposited onto the mold. The mold with water layer is then

degassed at 50 Torr for 20 seconds, followed by dipping in a sonication bath for 1 minute, resulting in all the micro-features being fully covered and penetrated by water. All the steps are conducted at room temperature. For this step, it is important to note that corona surface treatment on PDMS mold can decrease the contact angle and help degassing; however, induced hydroxyl groups on the surface of PDMS results in undesired bonding of the mold with the cast material.

For the second step, since the water precursor layer has already purged all the gas bubbles in the mold, a gelatin solution loaded with bio-active agents can be added, allowing it to mix with the water in the mold. This solution is prepared by mixing 100 µL of plasmid-Lipofectamine® LTX complexes solution (lipoplexes) with 100 µL of 5% w/w porcine gelatin solution. The mixed solution is then drop-casted onto the water loaded mold for dehydration. Vacuuming is avoided, since it can result in early gelation at room temperature (< 37 ºC), thus trapping bubbles (due to vacuum), resulting in microneedles that are too porous and brittle. The solution is instead dried at atmospheric pressure in a 37 ºC environment. At this constant temperature, the gelation rate of the mixed solution is delayed, allowing a gradual mixing of gelatin with the pre-degassed water over the initial several hours. After 1 hour of dehydration, another 100 µL of 10 % w/w porcine gelatin solution is added to the previously deposited gelatin layer for further dehydration. The polymer micro-structures are formed after complete dehydration without the need for applying pressure, vacuum, or high temperatures. The desired final thickness of the gelatin can be controlled by continuous addition of gelatin solution. To achieve this, additional gelatin solution is deposited while the partially-dried gelatin solution is still in aqueous form; otherwise, any formed rough surface (at the liquid-solid interface) will house gas bubbles under the newly added solution. Finally, the fabricated gelatin microneedle patch is released from the mold.

Gelatin and lipoplexes solution preparation

Porcine skin gelatin is provided by Sigma-Aldrich. 50 mg of gelatin powder is added to 1 mL of water and dissolved at 35 ºC. Lipoplexes is prepared by mixing 50 µl of plasmid solution (containing 1µg of nls-EGFP [8]) with 50 µl of diluted Lipofectamine® LTX solution (containing 4 µl of the lipid). The mixture is then incubated at room temperature for 30 mins. All dilutions are made using Opti-MEM® I reduced serum media from ThermoFisher Scientific. Nls-EGFP is a gift from Rob Parton (Addgene plasmid# 67652). Before casting, the two solutions are gently mixed to avoid the generation of gas bubbles.

EXPERIMENTAL RESULTS

Figure 2 presents microscopic photographs of the pre-fabricated PDMS mold, Figure 2(a), and the final cast gelatin microneedle array, Figure 2(b-c), showing the successful molding of slanted structures. The radius of curvature is measured to be 17 µm via the scanning electron microscopy (SEM) image. Previous investigations with microneedles have shown that similar sizes (i.e., radius of curvature ~10 µm) is sufficiently small to enable piercing of the epidermis for vaccine delivery [9]. The small ridges shown in the picture are due to the roughness of the original 3D printed microneedles (limitations of current state-of-the-art 3D printing).

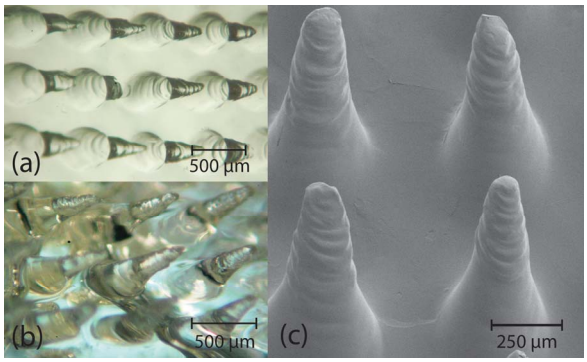

Figure 2: (a) Photograph of the PDMS microneedle mold; (b) molded gelatin slanted needles; (c) SEM image of fabricated microneedles.

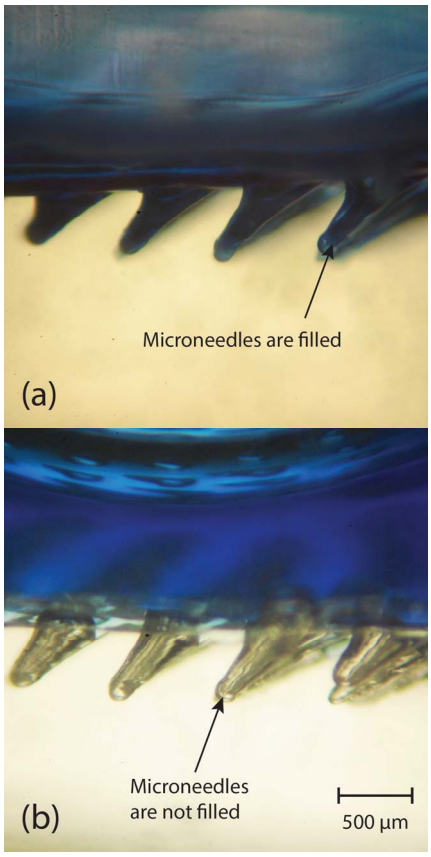

Figure 3: A comparison of cross-sections of PDMS mold with dyed gelatin solution when different molding techniques are used; (a) with the water pre-filling step the needles are filled completely; (b) without the pre-filling step, gelatin solution is directly casted onto the mold but does not fill the micro-patterns.

Figure 3 confirms the importance of the water priming step prior to the addition of gelatin. In Figure 3(a), microneedle molds are completely filled due to the use of water priming; in contrast, Figure 3(b) shows the results of not using a water priming step. Here, complete filling is not

achieved since the contact angle of pristine PDMS is 109.3º (hydrophobic), thus trapping the solution on the inlet of the microneedle cavity due to high surface tension.

A critical factor of this process is temperature control during dehydration, the temperature should be maintained at 37 ºC. If the temperature is below 37 ºC, cooling and gelation of the mixed solution will prevent the mixing of subsequently-added gelatin solution into the water as gelatin is known to be dissolved in water above 37 ºC. On the other hand, higher temperature induces conformational changes of DNA macrostructure [10], which leads to a loss of efficacy of vaccine and no transfection can be detected when tested on cells. At 37 ºC, the dehydration process can last for several hours, and during dehydration, more gelatin might be required to adjust the final microneedle patch thickness (as desired). The additional gelatin should be added before the gelatin in the mold is solidified. Thus, it is important to characterize the dehydration rate of gelatin and its thickness to be able to tune its final dimensions.

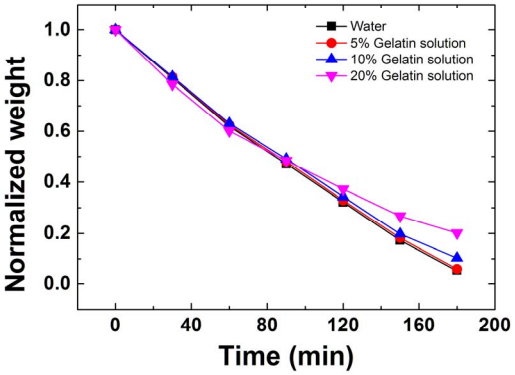

Figure 4: Normalized weight change of different concentrations of gelatin, due to dehydration at 37 ºC, in a cylindrical PDMS mold (2 mm height, diameter of 8 mm).

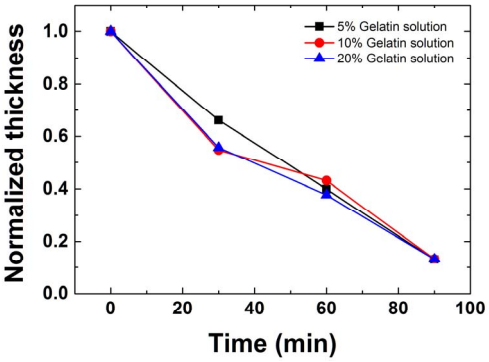

Figure 5: Normalized thickness change of different concentrations of gelatin, due to dehydration at 37 ºC, in a cylindrical PDMS mold (2 mm height, diameter of 8 mm).

Characterization of the dehydration rate is done via observation of the normalized weight change of gelatin solution. In the experiment, a cylindrical PDMS mold is used and the weight change of gelatin solution over time is measured. Figure 4 shows that the dehydration rate for

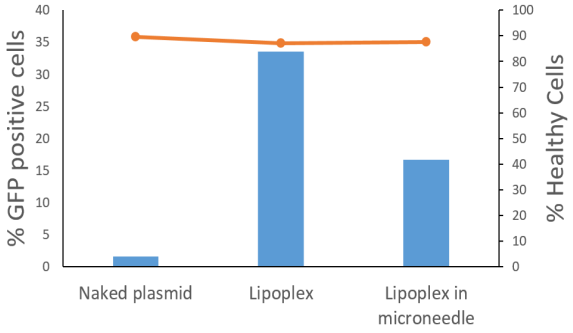

Figure 6: FACS analysis of transfected HeLa cells with AcGFP-N1 plasmid. Green fluorescent protein (GFP) is expressed by transfected cells and detected by flow cytometer. 1 µg of plasmid was applied for each group while naked plasmid and plamid-Lipofectamine® LTX complexes (lipoplexes) were negative and positive controls respectively. Tested microneedle patch was molded using gelatin solution mixed with lipoplexes.

differently concentrated gelatin solution varies slightly. Low concentrated gelatin solution has a slightly higher dehydration rate. As expected, the total dehydration time required depends on the amount of solution. When the mold size is 2 mm height and 8 mm in diameter, the total dehydration time for different concentration is about the same which is around 180 minutes.

Characterization of the thickness is conducted using the same cylindrical PDMS mold (2 mm height, diameter of 8 mm). The normalized thickness change of the gelatin solution is shown Figure 5. Gelatin is solidified when the normalized thickness reaches its minimum value (i.e., within 90 min, corresponding to 60% dehydration per Figure 4). At this point, introduction of additional liquid gelatin was observed to produce bubbles in the patch. Therefore, to avoid pores in the gelatin, any additional gelatin that is required (for patch dimension tuning) should be added prior to solidification of the gelatin in the mold (e.g., 60-80 minutes after the onset of dehydration). The process is iterative; so additional gelatin can be added every 60–80 minutes.

The transfecting capability of the gelatin microneedles was investigated to validate the fabrication process for use with bio-active components. Transfection was validated via FACS (Fluorescence Activated Cell Sorting) analysis on HeLa cells. HeLa cells were cultured in complete Dulbecco's modified Eagle's medium (supplemented with 10% fetal bovine serum). The cells are seeded at a concentration of 100,000 cells/well in a 24 well plate and incubated for 24 hours at 37^0C, 5% CO_2 and 95% relative humidity. Next, the complete media in each well is replaced by 1ml of Opti-MEM® media. The needle patch is added to a well for evaluating transfection efficiency of the released lipoplexes. Freshly made lipoplexes containing equivalent amount of plasmid and naked plasmid are served as positive and negative controls respectively. After another 24 hours of incubation, the cells are washed with Phosphate Buffered Saline (from Sigma-Aldrich), trypsinized and analyzed using FC500 flow cytometry analyzer. It is to be noted that the patch

dissolved within 15 minutes of incubation, releasing the entrapped lipoplexes. As shown in Figure 6, the *in vitro* validation shows a transfection in 16.7% of cells whereas a transfection in 33.5% of positive control group.

CONCLUSIONS

We have developed a micro-molding technique for casting biodegradable micro structure with hydrophobic elastomeric mold. The process improves current solvent molding method by incorporating the addition of water prior to the injection of material, resulting in a complete removal of residual voids in the structure. The process secures the therapeutic efficacy of delicate bio-agents from being compromised. The entire volume of cast material can be conviently controlled by following appropriate material addition timing protocal. As a proof of concept, microneedles loaded with lipoplexes were fabricated with curvature at the tips as small as 17 μm. The *in vitro* test confirmed that the bio-activity of plasmid can be conserved through the presented process.

ACKNOWLEDGEMENTS

The authors thank the staff of the Birck Nanotechnology Center for their support. Funding for this work was provided in part by NextFlex Project Call 1.0.

REFERENCES

[1] M. W. Ashraf, S. Tayyaba, and N. Afzulpurkar, "Micro Electromechanical Systems (MEMS) based microfluidic devices for biomedical applications," *Int. J. Mol. Sci.*, vol. 12, no. 6, pp. 3648–3704, 2011.

[2] G. Voskerician, M. S. Shive, R. S. Shawgo, H. Von Recum, J. M. Anderson, M. J. Cima, and R. Langer, "Biocompatibility and biofouling of MEMS drug delivery devices," *Biomaterials*, vol. 24, no. 11, pp. 1959–1967, 2003.

[3] Y. Xia and G. M. Whitesides, "Soft Lithography," *Angew. Chemie Int. Ed.*, vol. 37, no. 5, pp. 550–575, Mar. 1998.

[4] M. Ochoa, J. Zhou, R. Rahimi, V. Badwaik, D. Thompson, and B. Ziaie, "Rapid 3D-print-and-shrink fabrication of biodegradable microneedles with complex geometries," p. 1240443.

[5] J. H. Park, S. O. Choi, R. Kamath, Y. K. Yoon, M. G. Allen, and M. R. Prausnitz, "Polymer particle-based micromolding to fabricate novel microstructures," *Biomed. Microdevices*, vol. 9, no. 2, pp. 223–234, 2007.

[6] C. H. Ahn, J. W. Choi, G. Beaucage, J. Nevin, J. B. Lee, A. Puntambekar, and R. J. Y. Lee, "Disposable smart lab on a chip for point-of-care clinical diagnostics," *Proc. IEEE*, vol. 92, no. 1, pp. 154–173, 2004.

[7] M. M. Becker and Z. Wang, "Origin of ultraviolet damage in DNA," *J. Mol. Biol.*, vol. 210, no. 3, pp. 429–438, 1989.

[8] N. Ariotti, T. E. Hall, J. Rae, C. Ferguson, K. A. McMahon, N. Martel, R. E. Webb, R. I. Webb, R. D. Teasdale, and R. G. Parton, "Modular Detection of GFP-Labeled Proteins for Rapid Screening by Electron Microscopy in Cells and Organisms," *Dev. Cell*, vol. 35, no. 4, pp. 513–525, 2015.

[9] J.-H. Park, M. G. Allen, and M. R. Prausnitz, "Polymer microneedles for controlled-release drug delivery.," *Pharm. Res.*, vol. 23, no. 5, pp. 1008–19, May 2006.

[10] E. V. Hackl and Y. P. Blagoi, "The effect of temperature on DNA structural transitions under the action of Cu2+ and Ca2+ ions in aqueous solutions," *Biopolymers*, vol. 77, no. 6, pp. 315–324, 2005.

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