

Fabrication of Microneedle Using Laser Written PDMS Mold for Molecule Transport into Plant Skin

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Accepted 14 October 2009

Abstract

A laser writer was simply adopted to fabricate a PDMS microneedle mold having tapered cone tips for molecule or drug delivery. The advantage of the laser patterned PDMS mold was that it lacks of fabrication steps of a precedent master template, which requires many micro-fabrication steps. The laser fabrication had great efficiency with repeatability in manufacturing and simplicity in its process. By controlling laser power and writing speed, needle-tips having as small radius as 5 μm at vertex curvature could be fabricated. The length of the needle could range from 270 μm to 1500 μm . The biocompatible and biodegradable polymers of carboxy methylcellulose (CMC) and agarose were applied on the laser fabricated PDMS molds to cast microneedle replicas and tested their characteristics for a pore forming agent to plant skin. The results demonstrated the feasibility of successful application to a cost-effective manufacturing of microneedle and the availability of controlled transport of molecules, such as nutritions, for plant skin.

Keywords: Microneedle, Plant, PDMS (Polydimethylsiloxane), CMC (Carboxymethyl Cellulose), Laser writer

Introduction

Drug and therapeutic molecule for the clinical purpose of human beings are typically administrated orally as pills or capsules. Another common way of delivering drug is an injection by the use of a hypodermal needle which is made of a metal substrate¹. However, although the oral delivery or syringe needle delivery

would be advantageous, there can be limitations for an efficient delivery of drugs²⁻⁵. For example, the low bioactivity of biomolecules administered by the oral delivery is problematic due to enzymatic degradation and poor absorption in the digestive organ^{2,3}. In addition, with the intravenous injection through the hypodermal needle, pain, local damage and bleeding are accompanied^{3,4}. To overcome the difficulties with the conventional drug delivery systems, transdermal patches were invented²⁻⁴. The principle of the patch is a simple diffusion of drug across the skin. However, the method suffers from poor permeability to the skin and physiochemical problems such as skin irritations³⁻⁵.

Recently, there have been introduced an alternative delivery method, which is called as a microneedle, with a development of microfabrication technique¹⁻⁶. Microneedles are provided for a direct transport of molecules across biological barrier unlike the passive diffusion of drugs. Proteins, peptides, and vaccines can be adopted as a target substance for the microneedle delivery¹⁻³. With microneedle of 150 μm length, it was reported that four orders of magnitude increase in skin permeability was attained compared with the oral delivery². So far, microneedles have been prepared by (1) shaping the microneedles directly, (2) carving or etching a mold and then filing the mold to form the microneedle replica of the biomolecules, (3) micro-fabricating a microneedle master, using the master to make a mold, and then filing the mold to form the microneedle replica¹. However, the fabrication of microneedle have been often accompanied with complicated micro-fabrication processes including lithography²⁻⁵. For example, a bulk silicon wafer patterned by photolithography was etched with reactive ion etching (RIE) or etching chemical such as KOH. In addition, to have a sharpened tip vertex, special metal layer should be deposited, patterned and etched^{1,4}. Moreover, the typical processes were developed for the fabrication of a mold-master, which was a basic configuration for microneedle mold. Therefore, if a simple process without making of the mold-master is developed, it will be very advantageous.

At early stage, the microneedles had been introduced as a same method as an hypodermal injector having hollow micro-sized needles¹⁻³. They were reported that needles were fabricated from rigid metal or silicon and shaped as hollow for the delivery of liquid drug through their lumens. However, there are disadvan-

tages with the typical metal and silicon microneedles because there are possibilities to pose a problem if the microneedles are broken off in the skin^{4,5}. Therefore, biocompatible or biodegradable polymer substrate for microneedle will be more acceptable to be safely injected under the skin. So far, numerous methods to have polymeric microneedles have been developed using micro-fabrication processes^{4,5,7}. They include LIGA process, which is a simple laser process to have a mass production⁷. However, the microneedle by the LIGA process was for injecting liquid drugs and mimicked with a large-sized needle having a syringe. In addition, non-biodegradable poly methacrylate (PMMA) was used as the substrate⁷.

In recent reports, poly dimethylsiloxane (PDMS), which has been the most common mold substrate for microneedle so far¹⁻⁵, could be effectively ablated and fabricated by laser for biochip applications^{8,9}. By optimization of laser process, the shaping of the PDMS could be tuned to be used without significant problems.

Application of microneedle into plant skin or plant cell has been very limited so far. Typical applications of the microneedle were mostly dedicated to animal skin or human skin. However, with softer skin than the animal skin, the plant will be a desirable target organism to be used for the microneedle delivery. Even though the surface states of a plant depend on its functional tissue systems such as leaf, root, and stem, it is advantageous for the plant to accommodate the microneedle delivery that there is no rigid dead cell layer of stratum corneum like animal cells. The top surface of leaf skin is living cell layers of epidermis¹⁰. It is previously reported that there were several examples

of microneedles for plant. However, they were actually micro-capillary array to deliver genetic material such as DNA in a liquid phase¹⁰.

In this study, fine microneedles were designed and prepared by a simple process using laser writer. The microneedle was targeted to deliveries of drug, ingredient, and biomolecules into plant cells. Carboxy methylcellulose (CMC) and agarose solution was applied on the laser fabricated PDMS molds to form solid microneedles. The results demonstrate the feasibility of using laser writer for successful application as a cost-effective process. The microneedle can be used to enhance the ability of plants to absorb nutrients. Also, the microneedle can be a drug delivery system to protect crop or vegetable pathogens.

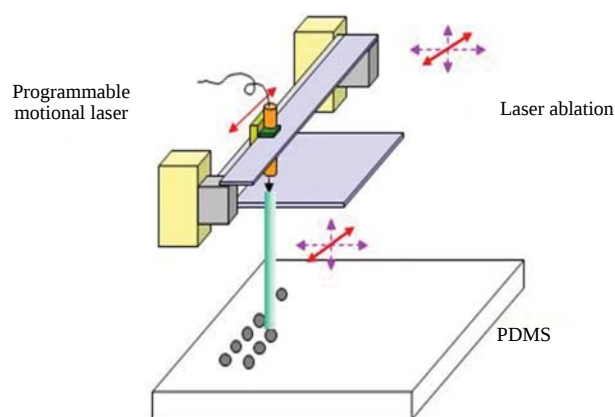


Figure 1. Schematic diagram of fabrication of PDMS mold using laser writer.

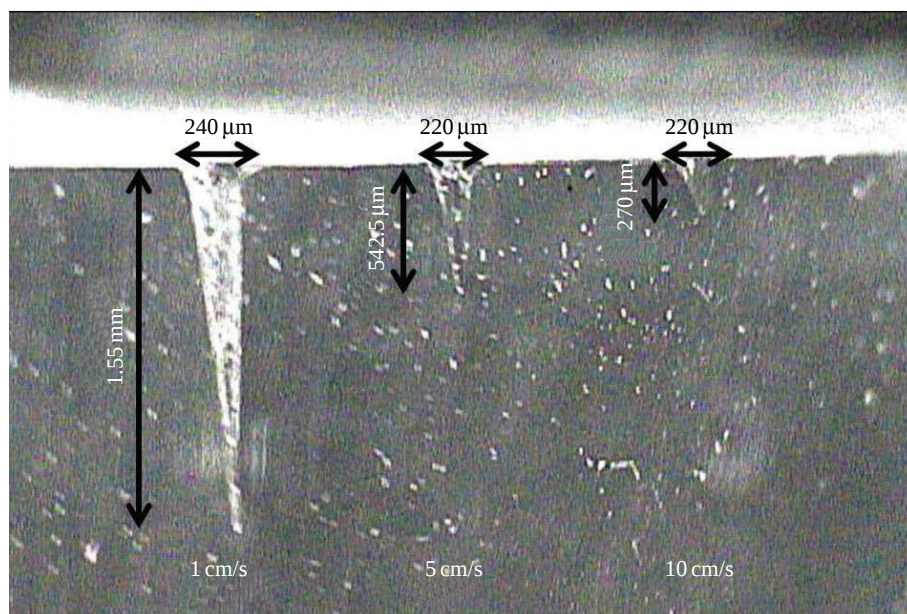


Figure 2. Optical images of PDMS micro-holes using laser writer by beam speeds at 10 cm/s, 5 cm/s and 1 cm/s.

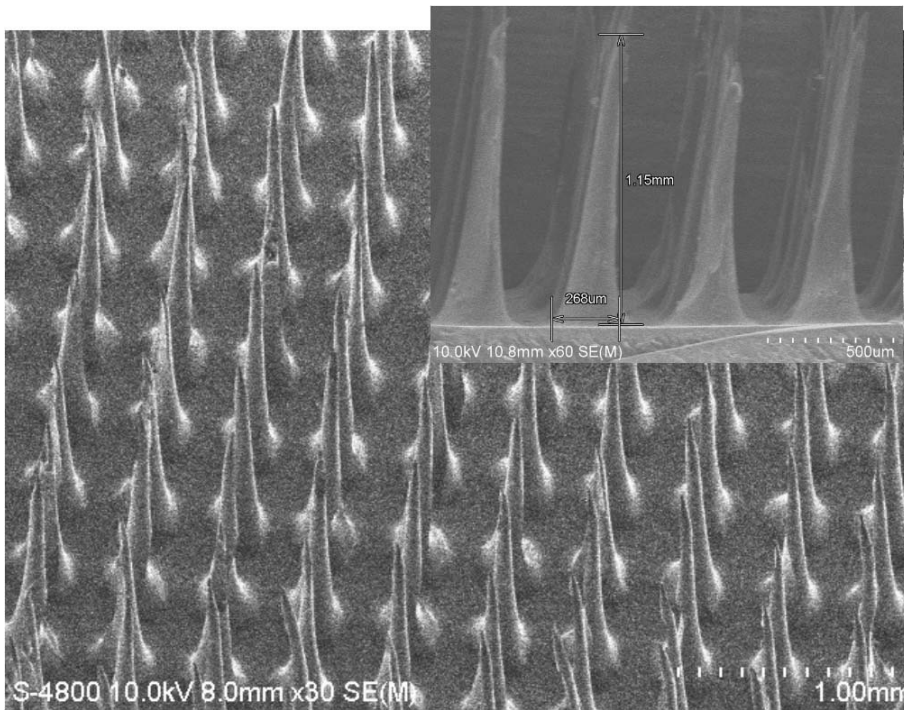


Figure 3. SEM images of CMC casted microneedles having density of 400 needles in an area of 1 cm² with length of 1.15 mm with aspect ratio of 4.3.

Results and Discussion

Fabrication of Microneedle Mold

Figure 1 shows the schematic diagram of fabrication of PDMS mold using laser writer. Laser beam was directly irradiated on the flat cross-linked PDMS substrate to have microneedle patterned micro-holes. Power control setting as well as focus control of the laser beam determined actual power of laser intensity. Also, conditions to have smoothly ablated PDMS surface could be obtained. Depending on variations in programmable drawing to write on the PDMS, smoothly tapered cone needles or twisty tapered cone needles could be fabricated (data not shown).

Figure 2 shows optical images of PDMS mold fabricated by the condition of beam to make various lengths of micro-holes. The depths were changed according to moving speed of beam, such as 1 cm/s, 5 cm/s and 10 cm/s. Depending on the speed of beam, depths of holes were 1550 μm, 542 μm, and 270 μm. The length could be controlled to be longer than 1550 μm. Since the skin surface of animal or plant is not flat due to dermatoglyphics, such as tiny wrinkles, the length should be long enough to guarantee neat penetrations². At the same time, the length could be reduced down to 150 μm with the same sharpness (data not shown). Conventionally, microneedles were fabricated with photolithography and etching processes having a straight cylindrical shaft^{1,4}. Therefore, additional process

to have a tapered shape or beveled shape were needed. In fact, in order to make a tapered cone microneedle master, it was reported that a glass substrate should be etched to have hemispherical microlenses at first. Then, using the microlenses, a special photolithography process with SU-8 should be accompanied with a precise alignment of UV exposure⁴. However, interestingly in this study, the tapered holes on PDMS mold were easily formed with achieving sharp tips for microneedle by simple adjusting of laser writing conditions. For the laser ablation, it is known that the basic ablation rate of micro-holes for microneedles could be determined by the total fluence of the laser^{8,9}. Therefore, more detailed investigations with the fluence will be required to have a perfect control of boring the microholes.

Figure 3 shows the SEM images of CMC casted microneedles from the laser fabricated PDMS mold. It could contain 400 microneedles in an area of 1 cm². By changing drawings in the program, denser microneedles than 400 needles/cm² could be obtained (data not shown). Density of microneedles can be important or not depending on the target skin. For example, for the penetration across stratum corneum of porcine or human being, dense microneedles were not helpful due to its sponge-like property. In this case, their sharpness was most important^{2,6}. The SEM image in Figure 3 also confirms a long length of 1550 μm with a high aspect ratio of 4.3.

Figure 4 shows the sharpness of casted CMC micro-

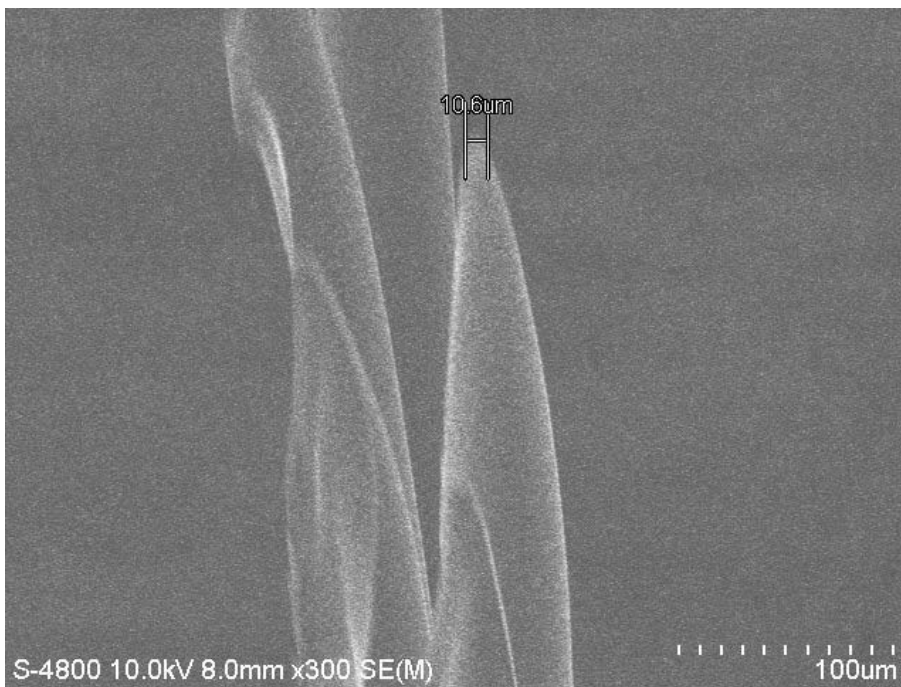


Figure 4. SEM image of sharpness of CMC microneedle casted on the PDMS mold.

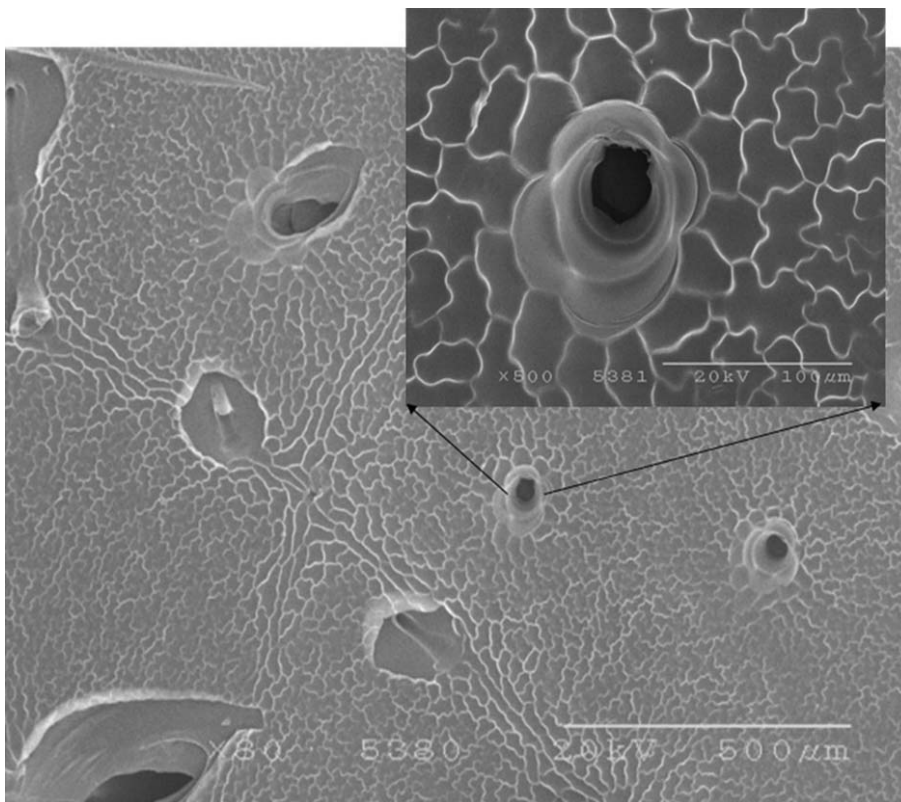


Figure 5. SEM image of pierced plant surface of cucumber leaf by agarose microneedles with an enlarged hole image.

needles. The microneedles have sharp tips with radius of curvature 5 μm. Since the plant cells are usually big enough to have a size of larger than 10 μm, the

radius size of 5 μm of the sharpened tip is allowed to have neat piercing and penetration into plant skin. The radius of tip was also controlled by the number

of laser irradiation as well as the beam speed. It indicates that the sharpness is controlled by heat transferred to the tip-end. PDMS has such a characteristic of transformation by thermal energy that it can bore micro-holes, which can be tapered cone shaped. Remnant from ablated PDMS has accumulated as black dusts.

Penetration across Plant Skin with Agarose Microneedles

From many reports, several issues were being investigated to improve the usage of microneedles. At the first place, a novel method for more efficient penetration through skin is required⁷. In this regard; an intensive application of microneedle to transport of molecule for plant is very acceptable because the skin of the plant is relatively weak for the penetration of microneedle. Typically, the penetration of microneedle was often limited by viscoelastic nature of the skin of animal⁷. However, since the first layer for plant leaf is epidermis cell, application to the plant skin will not have many problems without thick layers of lipids or proteins like skin of animal.

To assess the ability of microneedles to create transport pathways across plant skin, an array of 3600 solid microneedles in an area of 12.25 cm² was pressed into plant surface and then removed. The length of microneedles was 270 μ m. The plant tested in this study was a cucumber leaf, which is a model for vegetables. Microneedles made of 5% agarose were applied on the cucumber leaf. Figure 5 shows a SEM image of penetrated skin surface of the cucumber leaf with an enlarged image of a hole. The array of the agarose microneedles were successfully penetrated and dissolved into the skin of the cucumber leaf. Some of microneedles were failed to neatly pierce across plant skin as shown in Figure 5. Bigger holes than the size of microneedles which were believed to be damaged or torn during the penetration with the microneedles are detected in Figure 5. The radius of neatly pierced hole was measured around 15 μ m in the enlarged hole image. Therefore, top portions of approximately 30-40 μ m long microneedles tips were penetrated and dissolved. However, severe shear-induced breakages of microneedles without penetrations were not observed with the agarose needles. In addition, it was hard to check if there was fetal damage to plant, but, at least, no side effect was found after the injection.

Conclusions

PDMS mold for fabrication of microneedle could be easily fabricated with a laser writer. The tapered

cone-shaped microneedles with top vertex's radius of 5 μ m were successfully made with the PDMS mold, which was filled with 5% CMC and 5% agarose. The agarose microneedle was applied to cucumber skin to be easily penetrated and dissolved. The laser fabricated PDMS mold and its microneedles can be extensively used for transport of molecules into more rigid skins than the plant skin.

Materials and Methods

PDMS Substrate Preparation

PDMS (Sylgard 184, Dow corning) mold was made by concocting with polymeric PDMS solution (DC-184A) and hardener (DC-184B) for 10 : 1 ratio. The PDMS pre-solutions were poured on flat surface and the film was put to vacuum desecrator to remove any possible blisters completely. It was performed in atmosphere for 30 minutes and vacuum state for 30 minutes. Then, the film was heated and crosslinked in dry oven at 72°C for two hour before laser processing.

Fabricaiton of Microneedle Mold Using Laser Writer

To serve as molds for subsequent fabrication of microneedle structures, the hardened PDMS layer was shaped by laser ablation. The programmable CO₂ laser (Infrared light) writer (PL-40K, Korea stamp, Korea) was used to make tapered cone holes on PDMS mold. The laser beam was focused with a lens (f=30 mm) onto the PDMS surface. The programmable laser writer could change its power at 40-50 Watts generating thermal energy⁹.

Figure 1 is a schematic diagram for laser processing. In this study, speed and power of laser could control radius, depth, and top-edge size. The geometric characteristics were also controllable at program drawing step. Remnants from laser ablation could be washed by ultrasonic cleanser for 10 minutes with acetone and isopropylalcohol (IPA). Not otherwise specified, each hole for microneedle was patterned into 20 by 20 arrays of 220-240 μ m diameter with 450 μ m center-to-center spacing using the drawing program.

Casting of CMC and Polyacrylamide Solution on Microneedle Mold

Carboxy methylcellulose (CMC) has been used wider food industry and medicine department from high viscosity, thickening agent, stabilizers, suspended concentrate as anion cellulose derivative. We used mixing CMC powder and DI water. It was mixed more than 6 hours at room temperature to make 5% CMC solution. The 5% CMC solution was placed into PDMS mold.

Then, a vacuum was applied to fill the channels in the microneedles mold. The solution had low mobility due to very high viscosity. To make a nonporous solid microneedle, the entire mold was put inside of vacuum dessicator. Then, the whole unit was centrifuged by 6000 rpm for 10 min. To the resultant CMC microneedle, heat was applied more than 1 hour at 82°C. Dried CMC microneedle was baked to be completely hardened. The CMC microneedle could be easily removed easily from the PDMS mold. To make agarose microneedle, 5% agarose solution in DI water was placed into the PDMS mold and allowed to be dried for several hours. The drying process was repeated 3-4 times to have a concrete shape of microneedle. SEM images for microneedles were obtained with FESEM (Hitachi S-4800, Japan) with magnification of 30-120.

Acknowledgements

This work was supported by 2007 Research Fund of Myongji University.

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