공학석사학위논문

3D 프린터를 활용한 플라스틱 소재의 미소유체소자 마스터 몰드 제작

Plastic Masters from 3D Printed Models for Microfluidics

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Abstract

Plastic Masters from 3D Printed Models for Microfluidics

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We introduced an easily accessible and rapid fabrication method of creating non-planar microfluidic devices. By using the 3D printer and plastic molds, it was able to create polydimethylsiloxane(PDMS) devices without any cleanroom facilities or expensive equipment which were usually required in the conventional microfluidic device fabrication process. It is necessary to create endurable plastic master molds than use the 3D printed molds for PDMS microfluidic devices. The proposed master molds are stronger than the 3D printed molds and are easily replicable. The master molds do not need an additional coating to mold PDMS, so the molds can be used to replicate

numerous devices. We have demonstrated creating a master mold for microfluidic devices and using it for biological applications by cell co-culturing, proving this technique to be a successful way to provide an easy way to replicate microfluidic devices which can be used in life sciences. By combining the advantages using the 3D printer, such as design flexibility, with the biological and mechanical properties of PDMS, we show a new potential for cost-effective, time-efficient fabrication techniques of versatile microfluidic devices.

Keywords: 3D printer, Plastic master mold, transfer molding, Microfluidics, PDMS

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Chapter 1. Introduction

Microfluidic chips have been used for chemical and biological analysis since the 1980s and have become an important element in the lab on a chip(LOC) configuration[1], [2]. Microfluidics has advanced as the LOC designs developed into integrating experimental processes into a portable, automated device[3]. Technology for manipulating fluids on a chip have progressed and PDMS microfluidic chips have been used widely in many applications, such as encapsulating[4],[5], micro mixing[6], micro separating[7], [8], biological and chemical analysis[9], micro emulsifying[10], and so on. Among the polymers which have been used for microfluidic chips, PDMS casting is still the most popular material on the basis of its biocompatibility, transparency, cost, ease in manufacturing, and other favorable properties. [11]–[13]

Master mold for rapid prototyping of the microfluidic chips usually require photolithographic process which can create fine features in submicron resolution [10]. However, photoresists used in the process have a limited casting life time due to delamination and requires expensive equipment with cleanroom facilities. Also, photolithography is mainly used to create planar structures, but process for non-planar structures can be long and costly.

Current microfluidic designs have been increasing geometric complexity as it opens a potential of creating a more versatile platform for experiments. For example, 3-dimensional designs can create compact devices combined with active components such as valves and mixers. [15]–[17] In addition, it can create a more

realistic platform in biological applications when experimenting with cells. Therefore, more laboratories are searching for an easier fabrication process to create 3D master molds for PDMS microfluidic chips. From processes which require additional professional techniques such as micromachining[18], injection molding[19]–[21], etching[22], [23], Shrinky-Dink[24], [25], many options are considered for rapid prototyping of versatile PDMS microfluidic chips.

With the aforementioned techniques, 3D printer technology offers high topography flexibility and grants broader access to the fabrication of non-planar microfluidic chips.[26] Because of its efficiency, easy to access, cost-efficient, time-saving, accuracy, and productivity, scientists have applied this technology to create multidimensional devices.[27]–[30] However, there are difficulties related to making microfluidic devices with PDMS using the 3D printer and this has slowed down the merging of 3D printing technology and life science.

3D printers are dominantly used to create prototypes, but fabricating multiple replicas of PDMS microfluidic devices are difficult due to the mechanical properties of the materials. 3D printed master molds cannot be directly used to cast PDMS microfluidic devices because the silicone cannot be fully cured on the surface. Current techniques solve this problem by coating the 3D printed mold with paint air brush[31], Vaseline spray[32], silane vapor[33], and parylene coating. However, it is difficult to uniformly coat the mold and the coating delaminates after repeated demolding. In order to create multiple PDMS devices with high throughput, it is important to make as many master molds as possible and that is difficult with only a 3D printer.[34]

In order to solve these problems, we aim to introduce a robust molding method

using a 3D printer which can fabricate PDMS microfluidic devices that can be used in any kind of laboratory. The materials for master mold are easily obtainable in the market and non-expert users can have easy access to all the products used in this process. By creating a sacrificial silicone mold from the 3D printed structure, multiple replicas of plastic master molds are fabricated. Because plastic master molds do not require additional coating on the surface to demold PDMS, this process can rapidly manufacture numerous microfluidic devices.

Chapter 2. Materials and Methods

2.1 Device fabrication

2.1.1 3D printer mold fabrication

The source file for the device was designed by using a computer-aided design(CAD) software installed in Windows(Siemens NX). The design was based on the cell co-culture chip which uses capillary-edge guided patterning from our group (Figure 2.1). The CAD designs were saved in a STL format and imported into the computer software(EnvisionTEC MAGICS) which was supplied with the 3D printer. After repairing the STL file with the software and importing it into the 3D printer, the device was printed after 1.5 hours.

After the printing, the leftover resin was washed away by spraying isopropyl alcohol(Dae Jung) and sonicating it for 30 seconds. The residual alcohol is dried out by a blow gun with nitrogen and post-cured in a UV chamber for 10 minutes. Supports connected to the device are broken off and the walls are sanded down for a smoother surface. Afterwards, the device is put in the parylene coating machine (FEMTO Science, LAVIDA 110). The coating process is crucial because the silicon cannot cure at the surface of the 3D printer without a thin coating(Figure 2.2a).[35]

2.1.2 Master mold fabrication

After the coating of the 3D printed device is finished, mix 3ml of part A and B of silicone (Moldstar Fast, Smooth-on Inc.) each. Mix both parts thoroughly, and pour the amount into the 3D printer device. Place the device on a hotplate of 90°C for the silicon liquid to cure in 10 minutes. Cool the device in a refrigerator or at room temperature and the silicon mold will easily peel off.

Prepare a dish with the bottom and walls coated in PDMS. Polyurethane does not adhere to silicone, so it is important to thoroughly coat the plate. Attach the silicone mold to the bottom of this plate by curing it with silicon or instant glue. Mix polyurethane (Smoothcast 300, Smooth-on) and the curing agent by 1:1 ratio in weight. Pour in the polyurethane and make sure that the liquid touches the bottom of the dish first in order to prevent bubbles trapping under the liquid. Place the dish at 90°C hot plate and wait 10-15 minutes for the resin to cure. For a fully cured mold, baking the mold for a few hours on a hot plate is recommended (Figure 2.2b).

2.1.3 PDMS device fabrication

The final microfluidic devices are fabricated out of polydimethylsiloxane (PDMS, Sylgard 184, Dow Corning) using soft lithography and molding. The master mold made out of polyurethane resin (Smooth-on Inc.). PDMS base and the curing agent is mixed in 10: 1 (w/w) ratio and is cast in master. Before thermally curing the PDMS prepolymer in the mold, the PDMS was degassed in a vacuum chamber for

20 minutes in order to get rid of the bubbles entrapping in the device. After that, it was placed on a hot plate set at 85 °C for 30 minutes. The PDMS device is separated from the mold and the slight distortions on the edge of the device were cut out with scissors. The PDMS device (Figure 2.2c) and glass were cleaned with a nitrogen gas air gun and residue-free tape (3M). Then, both pieces were treated with oxygen plasma for 1 minute and bonded together. The device needs to be hydrophilic for the patterning, so it must be made right before preparing the cells for loading in the device.

2.2 Cell Experiment

2.2.1 Cell culture

Normal human lung fibroblasts (LF, Lonza) with passage number 6 to 9 were cultured in Fibroblast Growth Medium (FGM-2, Lonza) and used. Human umbilical vein endothelial cells (HUVEC, Lonza) which were cultured from passage 3 to 5 in Endothelial Growth Medium (EGM-2, Lonza) was used. The cells were passaged until the density was 80% confluence in the dish. The humidified incubator which stored the cells was 37 °C and 5% CO2.

2.2.2 Cell seeding in PDMS device

Dissolve the bovine fibrinogen (Sigma) in phosphate-buffered saline (PBS,

Hyclone) and prepare 2.5mg/ml of a fibrinogen solution and syringe filter the solution. Supplement the aprotinin (0.15U/ml, Sigma) to the solution and mix it with the suspended cells. The suspended cells can be obtained by disassociating the cells from the culture dish by adding 0.25% Trypsin-EDTA (Hyclone) and applying physical impact on the dish. HUVECs and LFs are both suspended in 6 to 7 million cells/ml in the fibrinogen solution.

First, the empty gel solution was mixed with thrombin (0.5 U/ml, Sigma Aldrich) and then immediately inserted into the inner edge of the side reservoir. As the gel solution was inserted, the gel is patterned into the device following the edge of the device. Before introducing the second solution into the device, the first gel solution was left to clot for 5 minutes at room temperature. The HUVECs solution was mixed with thrombin and quickly inserted into the middle channel and the LFs solution was inserted into reservoirs on each side of the device. After the gels clotted, EGM-2 was inserted in the reservoirs and the cell media was changed every 48 hrs.

2.3 Immunostaining

The samples were washed once with phosphate-buffered saline (PBS, Hyclone) and fixed in 4%(w/v) paraformaldehyde in PBS for 15 minutes. Then it was permeablized using a 0.15% Triton X-100 (Sigma Aldrich) diluted in PBS solution for 15 minutes. Then, the sample was blocked with 3% bovine serum albumin (BSA, Sigma Aldrich) diluted in PBS for 1 hour. After washing the samples once with PBS, the samples were incubated for 4 days in 4°C w with mouse monoclonal antibodies

specific for CD31 (Alexa Fluor1647, clone WM59) from BioLegend diluted 1:200. The samples were placed on a slope and the direction of the slope was changed once a day in order the immunostaining of the samples to be more efficient. Afterwards, the samples were washed once and stored in PBS before imaging.

2.4 Imaging

For the 3D imaging of the micro blood vessels formed in the device, an IX81 inverted microscope(Olympus) was used. The whole-construct image of the vessels was captured with a confocal PMT detector with the FluoView FV1000 confocal laser scanning unit. Confocal images could be processed by IMARIS software (Bitplane).

2.5 Scanning Electron Microscopy(SEM)

The polyurethane molds, silicone molds, and silicone devices were cleaned with residue-free tape (3M) and nitrogen gas air gun to remove dust and residual parts. Then individual parts were spin-coated with a layer of platinum. The samples were attached to conductive carbon tabs on a standard flat mount. Images were acquired at 5 kV acceleration and magnifications of x30 were used.

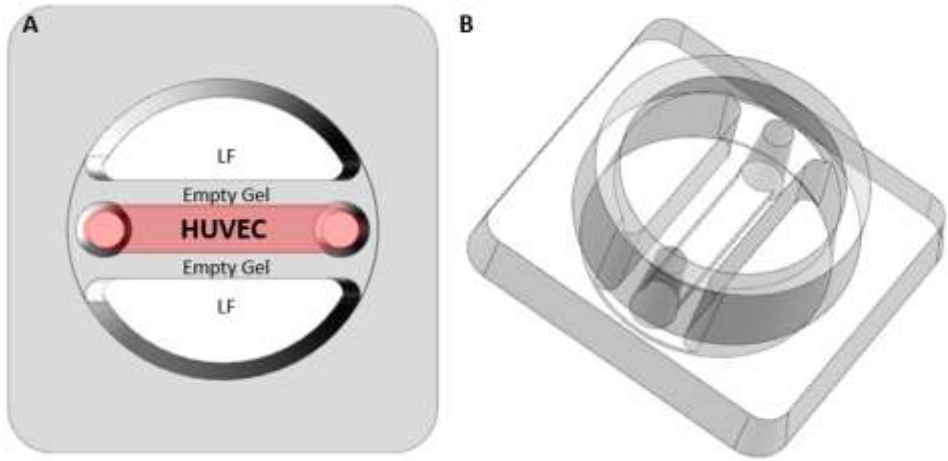

Figure 2.1 Schematic of cell co-culture device. (A) Bottom view of device with cell patterning (B) Top view of device

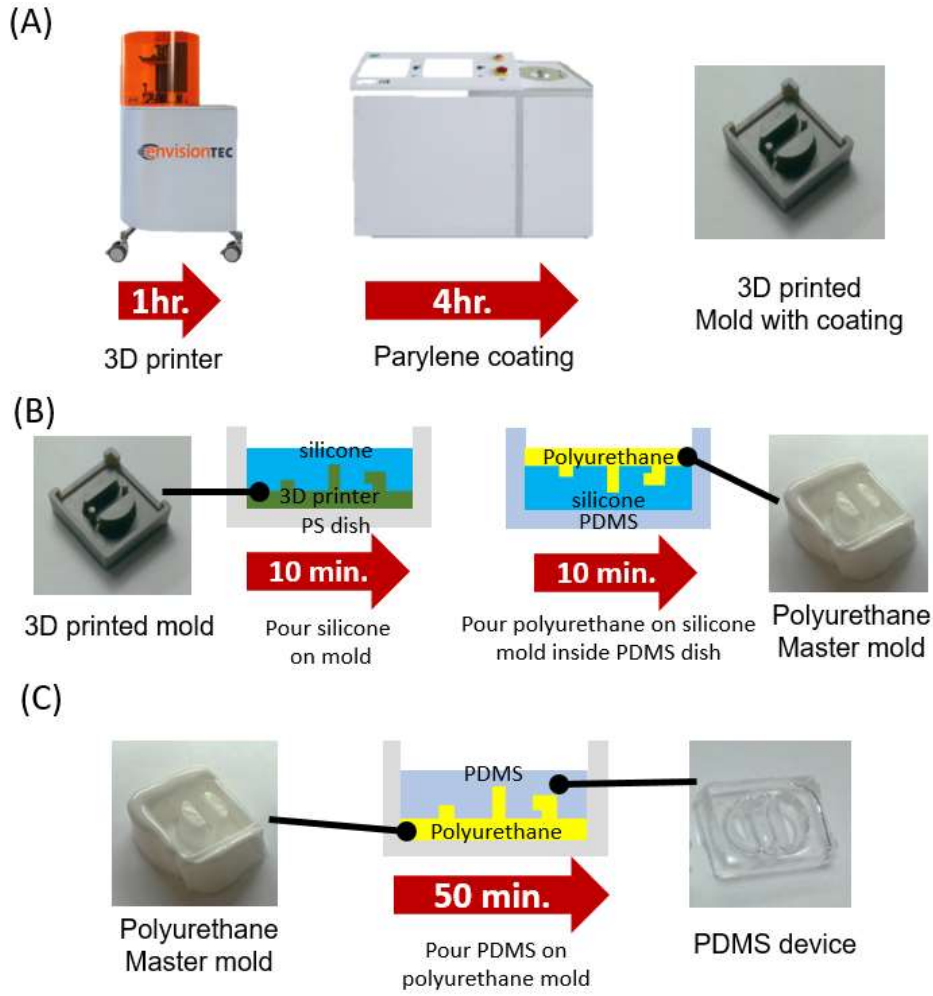

Figure 2.2 Fabrication process creating plastic master mold (A) 3D printed structure and coating process (B) Creating sacrificial silicone mold and plastic master mold (C) Fabricating PDMS device with plastic master mold

Chapter 3. Experiment Results

3.1 Plastic master fabrication

The plastic master mold is fabricated by creating a sacrificial silicone mold which is casted from the coated 3D printed structure (Figure 3.1). The high aspect ratio post arrays are more robust relative to the features in the 3D printer. Compared to the post arrays in created by photolithography, these long posts with a high aspect ratio are advantageous to fabricating the final PDMS device. This eliminates the process of making additional holes and ports in the device. By using this monolithic master, the tall and narrow posts are more robust and solid than the commonly used photolithography. The SEM images (Figure 3.2) also show the excellent quality of the reproduction from the 3D printer to the silicone molds and the transfer molding to the polyurethane molds from the silicone molds. The sharpness of the edges of the patterns and the walls could be examined from the images.

3.2 Cell co-culture chip and design

The microfluidic chip was fabricated by the 3D printing and rapid molding process introduced in this paper. By bonding the cover glass to the molded PDMS chip with plasma treatment, the device surface becomes hydrophilic which is crucial for capillary liquid patterning in the device. After bonding the device, cells must be seeded in the device in a few hours. The cells are patterned by capillary force guided

by the edges of the device. The spatially patterned cell co-culture of HUVECs and LFs progresses into vasculogenesis and can be replicated on other platforms (Figure 3.3).

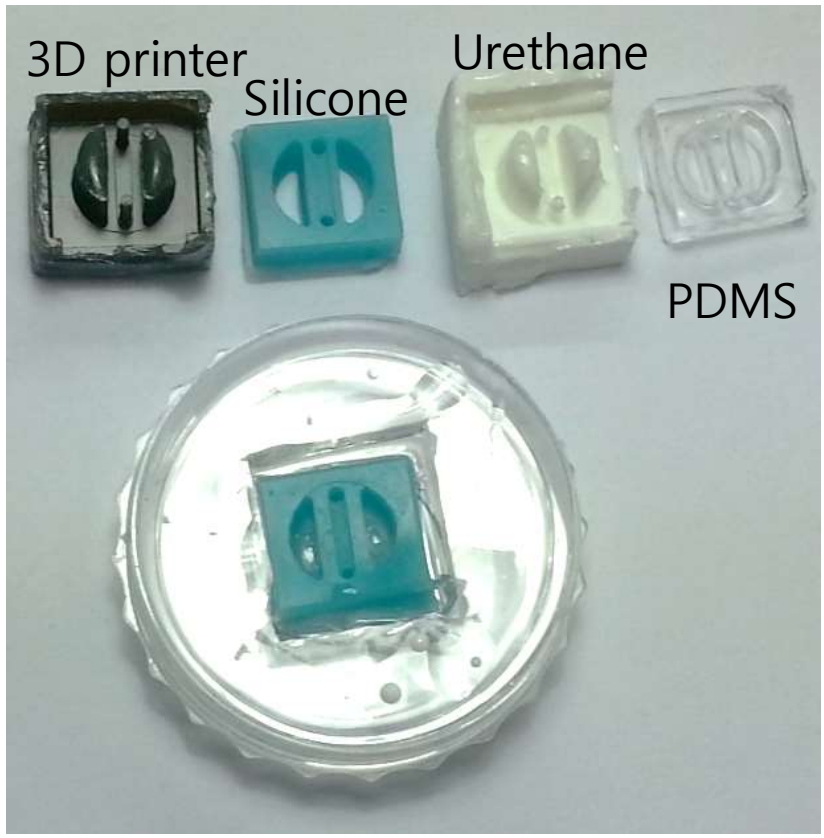

Figure 3.1 3D printed structure, silicone mold, plastic master mold, and PDMS co-culture device

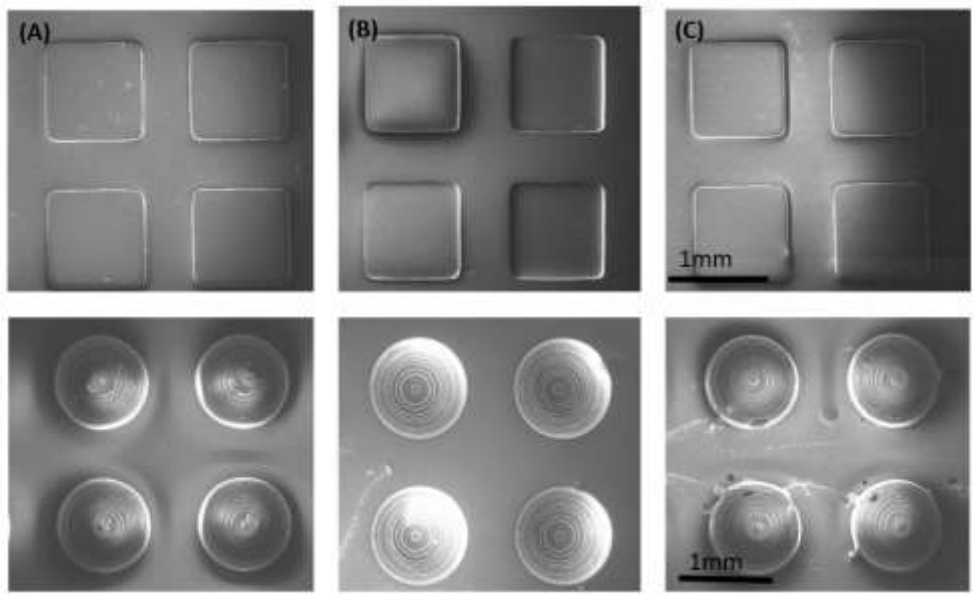

Figure 3.2 SEM image of (A) 3D printed structure (B) silicone mold, and (C) plastic master mold

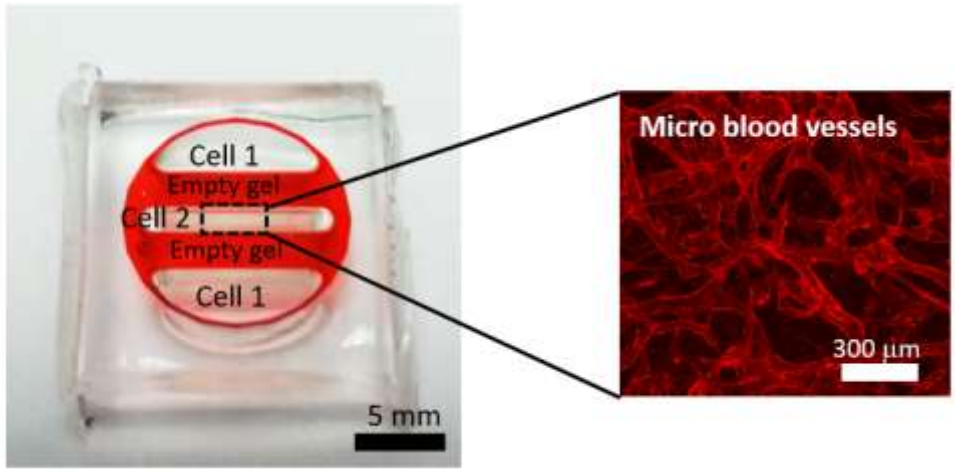

Figure 3.3 Capillary edge patterning on device(left) and cell co-culture image(right)

Chapter 4. Discussion

4.1 One step fabrication

Using the 3D printer, it has become possible to create a 3D device with the shape of the device made in one step. This means there is no need to cut out or punch holes for injection ports or reservoirs as the mold made from 3D printers create macro-sized templates and the holes can be made in one step. Not only does this method save time for fabrication as it cuts down on the time working on creating the device, but it also economical because there is almost no PDMS thrown away after the fabrication. In addition, accessories for tubing or connection to the pump can be simplified as the connection ports can be implemented into the microfluidic device, leading to a more simplified experimental process. Most of the holes and ports can be made during the fabrication process in one body and it drastically prevents the excessive use of PDMS.

4.2 Master mold fabrication

The time needed to create an additional master mold is about 20 minutes. Compared to the time needed to fabricate a new mold starting from the 3D printer, this process only takes less than half of what it would be without a master mold.

The difficulties in molding non-planar devices with complicated structures,

including undercuts or small and narrow posts with high aspect ratio, is the bubbles that are trapped in the edges or holes which are not clearly visible. This kind of defects may prevent the device from bonding to the glass or get in the way of liquid patterning. But with pre-vacuuming of the resin before use and cautious pouring of the resin in the mold can avoid these mistakes.

SEM images of the 3D printed mold, silicone mold, and polyurethane mold showed almost no shrinkage or difference in the surface quality. Also, the PDMS device molded from the polyurethane mold was successfully bonded to cover glasses, which proves the surface quality of the device. The master molds could also be self-replicated by using silicone molds and it has been demonstrated by the liquid patterning of these devices that the quality of the device has not deteriorated during the repeated replication process. This shows the high throughput of device replication leading to a highly efficient fabrication process for 3D PDMS microfluidic devices. Through working on creating solid master molds which can produce a large number of devices, this can lead to saving much more time in experiments and developing a product.

4.3 Biological applications

With the 3D printer, the freedom of design increases more than traditional methods. By adding an additional line for patterning or overhanging structure, the device can be used for more various types of cells and show a more accurate environment for cells. Future works will be concentrated on creating more complex designs to show there is no limit to creating molds for non-planar microstructures.

By creating more complex structures we can assemble a more accurate imitation of the biological environment for cell culture. The experimented device was limited to two types of cells, but by creating a multi-layered device we can pattern multiple types of cells in one device.

Current limitations of microfluidic chip design come from difficulties when peeling the PDMS chip from the master mold. Undercuts or complex 3D channels such as spiraling or intertwining channels are difficult to fabricate with conventional methods.

Chapter 5. Conclusion

This paper demonstrates a method to rapidly fabricate replicas of a master mold from a 3D printed non-planar structure. We created multiple plastic master molds by using sacrificial mold made from silicone which cures in 10 minutes. This silicone mold was casted from the parylene coated 3D printed structure. A sacrificial silicone mold is needed because it is a crucial that two materials have different stiffness for efficient demolding. 3D printed structure was parylene coated as silicone only partially cures on the surface of the device without the coating. This coating is not permanent so the silicone mold is used for creating replicas of the plastic master mold. The illustrated method can quickly create plastic master molds that are reusable and can be reproduced in at least 20 minutes. This process drastically speeds up the experimenting process and improves the optimizing process of developing a device design. The proposed technique will help researchers to fabricate many PDMS prototypes for experiments in short time only with a 3d printer leading to accelerate research and development.

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초 록

본 논문은 빠르고 쉽게 3차원 PDMS 미소유체소자를 제작하는 방법에 대한 내용을 기술하였다. 기존의 미소유체소자 제작방식과는 다르게 값비싼 장비나 청정실을 사용하지 않고 3D 프린터와 플라스틱 몰드를 이용하여 PDMS 미소유체소자를 제작하였다. 다수의 PDMS 미소유체소자를 빠르고 쉽게 제작하기 위해서는 3D 프린터 몰드를 바로 사용하기보다 플라스틱 마스터 몰드를 사용하는 것이 좋다. 플라스틱 몰드는 3D 프린터 몰드보다 견고하며 복제품을 제작하기 쉽다. 플라스틱 마스터 몰드는 3D 프린터 몰드와는 달리 추가적인 코팅을 필요하지 않으며 몰드의 복제품도 쉽게 만들수 있다. 본 논문에서는 세포 공배양 미소유체소자에서 실험함으로써 생명공학적 분야에서의 활용 가능성을 보였다. 또한, 생물 분야에서 여러 디바이스를 제작하는 과정에서 사용 가능성을 보였다. 디자인의 자유도가 높은 3D 프린터의 장점과 PDMS의 기계적, 생물친화적 장점을 적절하게 조합하여 시간 효율적이면서 금액적으로 적절하게 미소유체소자를 제작할 수 있는 방법에 대한 가능성을 보였으며 다양한 분야에서 활용될 수 있을 것이다.

주요어: 3D 프린터, 플라스틱 마스터 몰드, 전이 몰드, 미세유체소자, PDMS

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