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FABRICATION OF MICROMACHINED MOLD MASTERS FOR 3-D, HIGH-ASPECT-RATIO CELL CULTURING SUBSTRATES

Yanzhu Zhao
School of Electrical and Computer Engineering
Georgia Institute of Technology
Atlanta, GA 30332, USA
gtg173c@mail.gatech.edu

Xiaosong Wu
School of Polymer, Textile and Fiber Engineering
Georgia Institute of Technology
Atlanta, GA 30332, USA
gtg367s@mail.gatech.edu

Yong-Kyu Yoon
School of Electrical and Computer Engineering
Georgia Institute of Technology
Atlanta, GA 30332, USA
yky7434@ece.gatech.edu

Jung-Hwan Park
Department of Biomedical Engineering
Georgia Institute of Technology
Atlanta, GA 30332, USA
junghwan.park@gmail.com

Stephen J Kennedy
Orthonics, Inc.
Suite L1312, ATDC Biosciences Center, 311 Ferst Drive
Atlanta GA 30332, USA
steve.kennedy@orthonics.com

Zvi Schwartz
Department of Biomedical Engineering
Georgia Institute of Technology
Atlanta, GA 30332, USA
zvi.schwartz@bme.gatech.edu

Barbara D Boyan
Department of Biomedical Engineering
Georgia Institute of Technology
Atlanta GA 30332, USA
barbara.boyan@bme.gatech.edu

Mark G Allen
School of Electrical and Computer Engineering
Georgia Institute of Technology
Atlanta, GA 30332, USA
mark.allen@ece.gatech.edu

ABSTRACT

In this paper, three approaches to micromachined mold master structures for molding of a wafer-scale bone culturing platform are compared and contrasted. The processes investigated are a silicon deep-reactive ion etching (DRIE) process, an SU-8/polydimethylsiloxane(PDMS) process, and a multi-step SU-8 process. Upon comparison of the advantages and disadvantages of each approach, a wafer-scale implementation of bone cell culturing substrates is successfully demonstrated using the two-step SU-8 process, and successful duplication of hydrogels based on these molds is demonstrated.

INTRODUCTION

Biomaterials play an important role in medicine today with a broad range of applications that include: medical devices, artificial implants, drug delivery coatings, and cell scaffolds for tissue regeneration. The material surface is expected to support cell colonization, migration, growth, and differentiation, and to guide the development of the required tissue. The important factors governing surface properties include surface chemistry, mechanical strength, surface structure, degradation kinetics and biological factors. Each tissue/organ needs a specific and individual design with appropriate material properties [1-4]. Recently, technologies based on micro-electro-mechanical system (MEMS) microfabrication have been used to fabricate matrices with high spatial resolution of both surface and internal structures [5-7]. To better mimic the natural biological environment, it is often desirable to achieve three-dimensional surfaces using high-aspect-ratio micromachining. Micromolding is an attractive approach to achieve complex 3-D surfaces at low-cost and high-volume, with the added advantage that both microtexturing and the use of tissue-friendly materials are enabled. One of the key steps for successful micromolding is fabrication of a suitable mold master. A special challenge exists where some cells have not only 3-D structural requirements, but also a high-aspect-ratio structural demand to implement microscale grooves or dimples on the substrate surface for efficient biological interfacing. Further, the molding of many biological materials may need aggressive processes such as repetitive freezing and thawing cycles, placing severe robustness requirements on the mold masters [8-10].

Several candidate processes for the fabrication of robust mold masters can be considered. First, silicon deep-RIE is a standard bulk fabrication technique, and it is capable of making high-aspect-ratio structures up to 1:20 [11]. Micromolding using PDMS as a mold material is also widely used as a high-aspect-ratio fabrication technique [12, 13]. In addition, SU-8, an epoxy-based negative ultra-thick photoresist, has been widely used for high aspect ratio applications because of its very low absorption in the near UV range. SU-8 can also be used for the fabrication of three-dimensional structures [14-17].

In this paper, three micromachined master structure fabrication methods are proposed and compared. The methods investigated are: a silicon deep-RIE process, an SU-8/ polydimethylsiloxane(PDMS) process, and a multi-step SU-8 process. The feasibility and utility of the two-step SU-8 process is successfully demonstrated by means of master pattern fabrication for a bone culturing mold that has very specific structural requirements to induce cell adhesion.

NOMENCLATURE

Cell Substrates, Micromachining, Micromolding

FABRICATION

Device Concept

The surfaces with high feature resolution are designed to provide a three dimensional environment for cell growth. Each surface consists of approximately 1900 micro wells within a circle of 15 mm diameter, where each well is shaped as a snowflake and its nominal diameter is approximately 150µm and depth 150µm. Each snowflake has salient patterns along with the sidewall in the size of 2, 5, and 10µm, and dimple patterns at the bottom with varying diameters and depths of 2, 5, and 10 µm. The salient sidewalls and the dimples are intended to stimulate cell growth as well as to provide better cell adhesion to the mold.

Deep-RIE Process

Silicon Deep Reactive Ion Etch (DRIE) is a standard bulk micromachining fabrication technique commonly utilized to fabricate high-aspect-ratio structures in silicon.

The silicon DRIE process is described in Figure 1. In this process, sequential two-step silicon DRIE etching is used to fabricate a negative form of the master, which can be converted to a column shape master by an additional molding step. First, a thin layer of positive photoresist (SC1813) is spin-coated (3000rpm, 40s) on an oxidized (1000 nm) silicon wafer, and the photoresist layer is patterned for dimple definition (Figure 1a). A DRIE process is performed to etch through the SiO_2 and into the underlying Si. The Si etching depth is 2, 5, or 10 µm depending on dimple diameter of 2, 5, or 10 µm, respectively. (Figure 1b). A layer of photoresist (SC1813) is spin-coated on this partially-etched wafer and is patterned to define the larger 'snowflake' pattern (Figure 1c). A second DRIE process is performed on the uneven surface, forming the negative snowflake/dimple structures. The etching depth is controlled to be 150 µm (Figure 1d). The etching rate for the Si was approximately 200 µm/hour.

The fabricated negative mold master is shown in Figure 2. During the second DRIE process, the overall pattern of the dimple structures is preserved to the depth of 150 µm. Since this two-level mold is made of a monolithic material, Si, it is mechanically robust and would be an appropriate master material for a subsequent micromolding process in a harsh environment. Also, this process can be extended to form additional layers in the silicon structure by defining and patterning additional layers during the etch process.

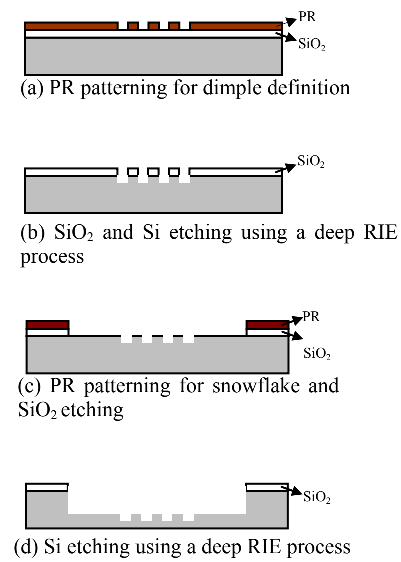
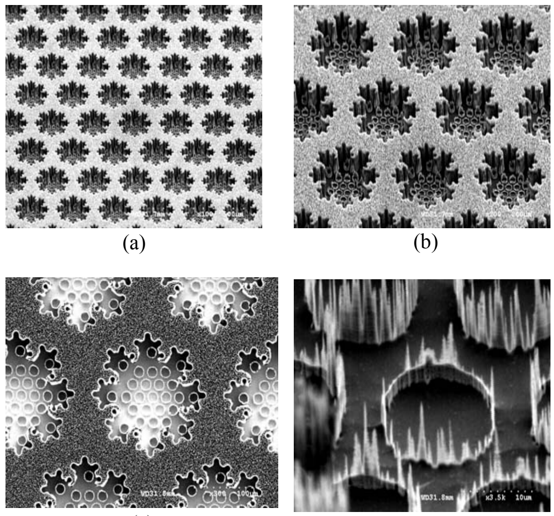


Fig. 1 Process flow of a silicon deep RIE process for robust multilayer mold masters

However, as shown in the magnified view of the dimples of Figure 2 (d), there are many micro spike structures around the edge of the dimples observed. This kind of micro grass often increases the contact area between the subsequent cast material and the Si mold, resulting in low yield during the replica separation stage. Although it is possible to define parameters to remove this grass or prevent its formation, its presence does reduce the process window for these types of structures. Thus, although this deep RIE process provides a straightforward solution for robust substrate fabrication in silicon, it was not pursued further.



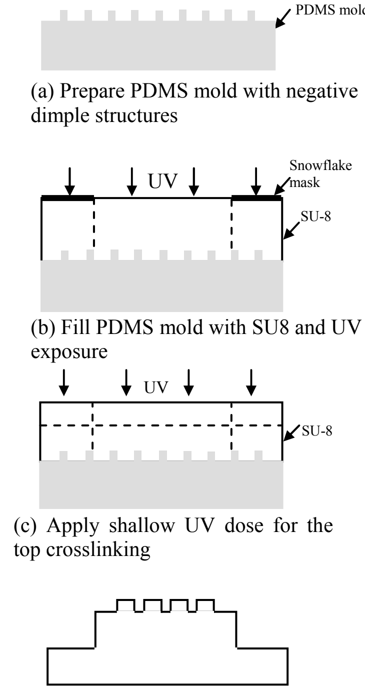
(a) (b) (c) (d)

Fig. 2 Fabricated mold master in silicon by a deep RIE process

SU-8/PDMS Process

Polydimethylsiloxane (PDMS), also known as silicone rubber, is a popular material choice for micromolding. PDMS is inexpensive, biocompatible, and can be easily bonded to itself, allowing the fabrication of multilayer structures.

In contrast with the DRIE molds discussed above, in which the mold negative was formed directly, the PDMS process involves the fabrication of a convex-shaped master first which is subsequently transferred to its negative. The fabrication discussed here is focused on the convex-shaped mold master patterning. The SU-8/PDMS process is detailed in Figure 3. First a dimple structure is made of SU-8 on a silicon wafer. Then a PDMS dimple mold is formed through micromolding on the SU8/silicon master: the PDMS monomer and curing agent are mixed in a 10:1 w:w ratio, degassed, poured onto the SU8/silicon master, and cured in an oven at 80^{o}C for 1 hour. For high-aspect-ratio mold topographies, this step may need to be performed under vacuum to remove air bubbles. Once polymerization is completed, the PDMS layer is carefully peeled off. The mold can be reused many times, which reduces the cost considerably (a). Then an amount of SU8 is poured onto this PDMS dimple mold and soft baked on a hotplate at 95^{o}C for 24 hours. Then this uncrosslinked SU-8 is exposed to form the snowflake structure (b). A subsequent weak exposure is performed from the backside as well to form a common substrate for all the snowflake/dimple structures (c). Finally the SU8 with molded dimple structures is separated from the PDMS mold and developed to form the final master device (d).



(a) Prepare PDMS mold with negative dimple structures

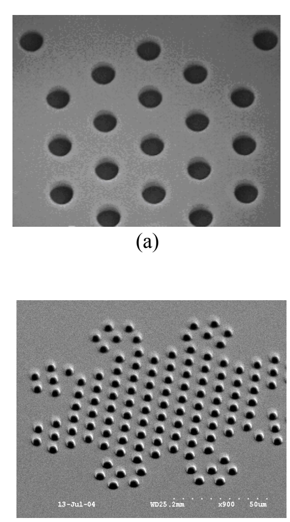
(b) Fill PDMS mold with SU8 and UV exposure

(c) Apply shallow UV dose for the top crosslinking

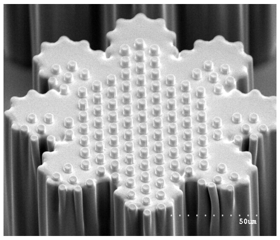
(d) Separate SU-8 from the PDMS mold and develop

Fig. 3 Process flow of SU8/PDMS process

This process uses a combination of structural molding and lithography together to form a unitary master structure piece, which ensures the necessary robustness for subsequent molding in harsh environments. Figure 4 shows a PDMS mold with dimple structures (a) and an uncrosslinked SU-8 sample molded from this PDMS mold (b). The desired 3-D structure could be achieved by further lithography of the uncrosslinked SU-8.



(a) (b)



(c)

Fig. 4 (a) PDMS mold with dimple structures (b) molded SU-8 before UV exposure (c) and fabricated SU-8 structure with 5µm features

However, it is difficult to avoid misalignment that occurs between the snow flake mask to the dimple pattern on the PDMS layer due to the coefficient of thermal expansion (CTE) mismatch of SU-8 and PDMS as shown in Figure 4c. So, although this approach could be utilized to provide high-quality mold masters for a relatively small area of the mold, this CTE mismatch issue remains a challenge to produce wafer-scale mold masters in this fashion.

Two-step SU-8 process

The third process is using SU-8 to make the masters. SU-8 is selected because of its wafer-scale fabrication ability and the robustness of high-aspect-ratio structures.

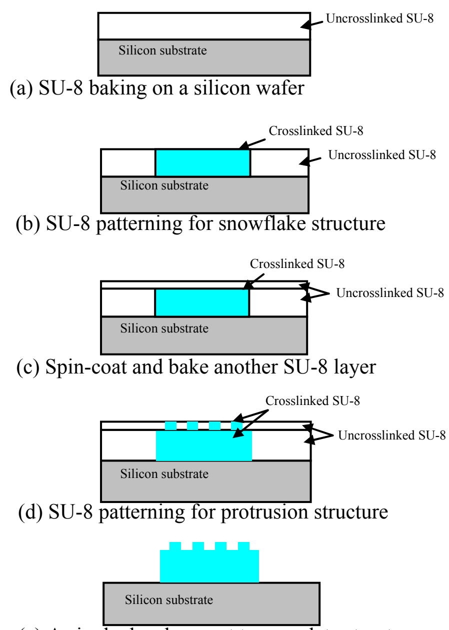


Fig. 5 Process flow of a two-step SU-8 process

A surface micromachined two-step SU-8 process is proposed and described in Figure 5. First, an approximately 150µm thick layer of SU-8 (Microchem, SU-8 2025) is spin-coated on a 4 inch silicon wafer (a). After soft baking, the SU-8 is exposed to form the snowflake structure, followed by a post-exposure bake to cross-link the SU-8 structure (b). After cooling down, instead of usual developing process, an additional thin SU-8 layer (2, 5 or 10µm thick) is spin coated on the first layer of SU-8 (c). After soft baking, the thin SU-8 layer is aligned and exposed to form the small extrusion structure, followed by post baking. (d). The two layers of SU-8 are developed in a single-development step to complete the master pattern (e).

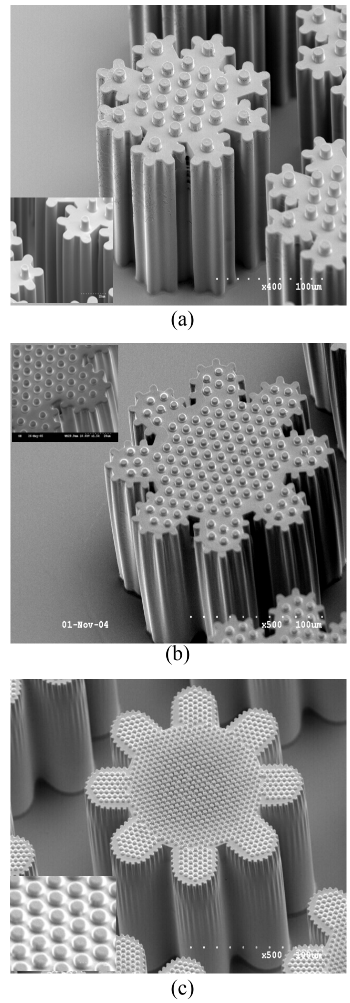


Fig. 6 Fabricated SU-8 master structures with (a) 10µm features (b) 5µm features (c) 2µm features

Both the convex snowflakes and dimples are made of SU-8, so the final device will be a single-body, unitary piece of SU-8 structure. The master structures will not suffer from mechanical mismatch problems such as delamination between the snowflakes and the extrusion parts. Further, misalignment due to CTE mismatch is eliminated enabling wafer scale fabrication. Figure 6(a)(b)(c) shows the successfully fabricated SU-8 mold masters with 10, 5, and 2 µm features, respectively.

Through this process, a successful wafer-scale implementation has been achieved. Figure 7 shows the different length scale of the SU-8 structures with the 5 µm feature structure. The process provides reliable patterning over the 5 order of length scale (from 100mm wafer size down to 2 ~10 μm).

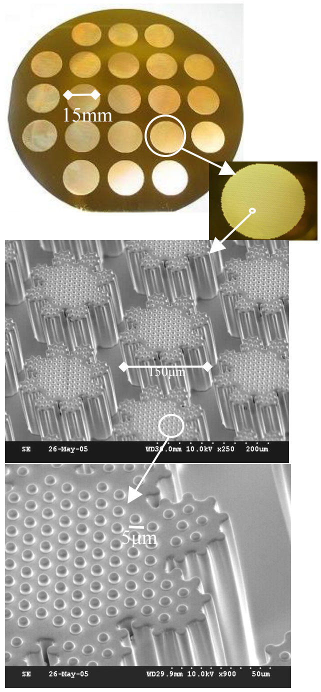
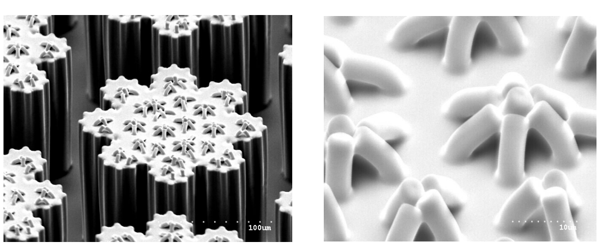


Fig. 7 Fabricated SU-8 master structures with 5μm features in a 4 inch wafer scale

In this process, control of both optical dose for the lithography and post-baking time is important. As an example, if the post-baking time after the first UV-exposure is too little, the first layer of SU-8 is not crosslinked completely, leading to unsuccessful extruded parts. It is also observed that when the baking time is too long, the top surface of each snow flake structure bows. When this occurs, the second SU-8 layer is not of uniform thickness, leading to collapsed structures as shown in Figure 8.



(a) (b)

Fig. 8 SU-8 master structures with collapsed 5μm structures due to the long baking time (a) lateral view of the snowflakes (b) detail view of dimples

The final cell culturing substrates can be formed directly from the fabricated master structure or alternatively from a mechanically more robust secondary master structure made from an additional micromolding process. A secondary master device made from polyurethane (PU) is shown in Figure 9. To increase the biocompatibility, the PU master is coated with gold.

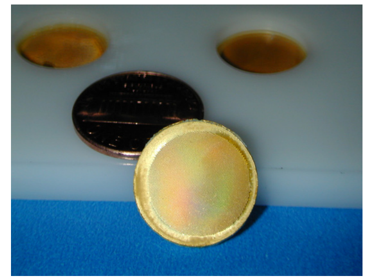


Fig. 9 A secondary mold master made from polyurethane (PU) coated with gold

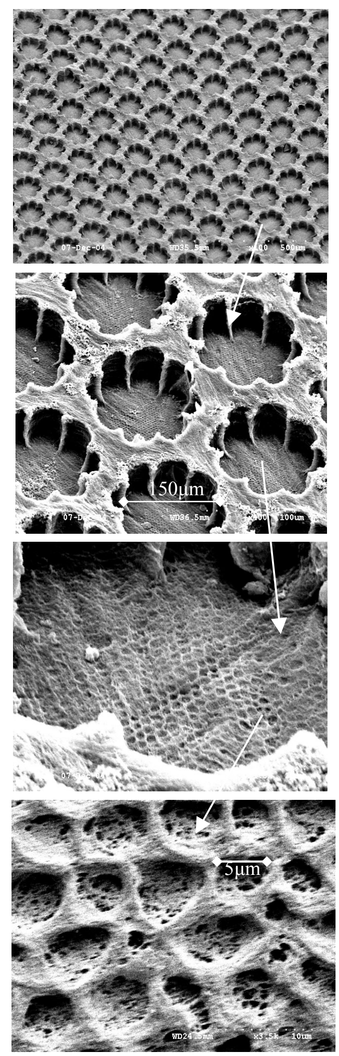


Fig. 10 Molded hydrogels from the SU-8 master mold

Figure 10 shows a SEM image (Hitachi 3500H SEM) of the final hydrogels formed by multiple freezing/thawing steps from the master after a super critical drying step (Tousimis Super Critical Dryer). Both snowflake and dimple structures with 5µm feature size are successfully transferred with high fidelity to hydrogel surface. The structural deformation is due to the critical drying step which is needed to perform a SEM imaging. The hydrogel containing the MEMS-molded features is now suitable for subsequent cell culturing.

CONCLUSION

Three approaches to micromachined mold master structures for molding of a wafer-scale cell culturing substrate are compared and contrasted. The mold master is for a wafer-scale bone culturing platform that has specific high-aspect-ratio and three dimensional requirements. The processes investigated are: a silicon deep-reactive ion etching (DRIE) process, an SU-8/ PDMS process, and a multi-step SU-8 process. The DRIE process is a standard bulk micromachining technique, the SU8/PDMS process combines the SU-8 micromachining and PDMS micromolding for 3-D structure fabrication, and the multi-step SU-8 process is a modified lithography process for thick negative photoresists.

Based on the experimental results, the advantages and disadvantages of these three approaches are summarized in the following table. Both the DRIE and the multi-step SU8 processes provide a wafer-scale micromachining solution for the mold masters, while the multi-step SU8 process has better yield. The SU8/PDMS process is a unique process that introduces PDMS micromolding into the common lithography process, thus providing more flexibility for the fabrication of complex structures. However, to achieve wafer-scale fabrication, this process needs to be further improved to address the CTE mismatch issue.

	Advantages	Disadvantages
Deep RIE process	Standard-bulk-micromachining High-aspect-ratio Robust and Wafer-scale-fabrication	Narrow process window
SU8/PDMS process	High flexibility for fabricating of complex structures	Difficult wafer-scale-fabrication due to CTE mismatch
Multi-step SU-8 process	Simple process with high yield High-aspect-ratio Robust and Wafer-scale-fabrication	Non-uniformity of small features

Upon comparison of the advantages and disadvantages of each approach, a wafer-scale implementation of mold masters for cell substrates is successfully demonstrated using the multi-step SU-8 process, and successful duplication of hydrogels based on these molds is demonstrated.

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