Biomed Microdevices (2014) 16:655–660
DOI 10.1007/s10544-014-9868-y

Fabrication of a three-layer SU-8 mould with inverted T-shaped cavities based on a sacrificial photoresist layer technique

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Published online: 23 May 2014
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Abstract A novel method for fabricating a three-layer SU-8 mould with inverted T-shaped cavities is presented. The first two SU-8 layers were spin coated and exposed separately, and simultaneously developed to fabricate the bottom and the horizontal part of the inverted T-shaped cavity. Then, a positive photoresist was filled into the cavity, and a wet lapping process was performed to remove the excess photoresist and make a temporary substrate. The third SU-8 layer was spin coated on the temporary substrate to make the vertical part of the inverted T-shaped cavity. The sacrificial photoresist layer can prevent the first two SU-8 layers from being secondly exposed, and make a temporary substrate for the third SU-8 layer at the same time. Moreover, the photoresist can be easily removed with the development of the third SU-8 layer. A polydimethylsiloxane (PDMS) microchip with arrays of T-shaped cantilevers for studying the mechanics of cells was fabricated by using the SU-8 mould.

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Keywords SU-8 mould · PDMS · Cast moulding · Sacrificial layer

1 Introduction

SU-8 is an epoxy-based negative photoresist, which has been specifically developed for making thick and high-aspect-ratio microstructures (Lorenz et al. 1997). Initially, SU-8 was used as an electroplating mould (Lorenz et al. 1997; Lorenz et al. 1998). In 1998, Whitesides' group developed a rapid prototyping method, in which SU-8 was first used as a mould to cast polydimethylsiloxane (PDMS) microchannels (Duffy et al. 1998). The SU-8 photoresist was spin coated on a silicon wafer, and a printed transparency was used as the photomask to make the mould. Now, this simple method has been widely adopted for replicating various PDMS microstructures (Chen et al. 2013a; Chen et al. 2013b). However, this single photolithographic process is limited for the fabrication of simple microstructures.

To fabricate complex microstructures, a wide range of new methods have been developed (Bertsch et al. 1999; Waits et al. 2003; Han et al. 2004; Kim et al. 2004; Wang et al. 2014). For example, Bertsch combined microstereolithography and UV lithography for fabricating three-dimensional (3D) SU-8 microstructures (Bertsch et al. 1999). Waits fabricated gradient-height microstructures by gray-scale lithography (Waits et al. 2003). Kim fabricated hollow SU-8 microstructures by a backside exposure method (Kim et al. 2004). Han fabricated 3D microstructures with inclined/rotated UV lithography (Han et al. 2004). To further increase the three dimensionality, multi-layer SU-8 complex microstructures were fabricated by multiple coatings and exposures followed with a single development step (Bohl et al. 2005; Xu et al. 2006; Zou et al. 2013). While this method is easy to operate, it is still challenging to build a width-increasing multi-layer SU-8 microstructure (e.g., T-shaped microstructures) or a multi-layer SU-8 mould with a width-decreasing cavity (e.g., inverted T-shaped cavities) since some portion of the unexposed area of the lower

SU-8 layer will be exposed if the upper SU-8 layer has a wider exposure area. Agirregabiria fabricated each layer separately, and then stacked these layers to make complex 3D multi-layer microstructures by an adhesive bonding method (Agirregabiria et al. 2005). Mata constructed multi-layer microstructures by controlling the UV exposure dosage of each layer (Mata et al. 2006). Ceyssens developed a built-in UV blocking metal layer method (Ceyssens and Puers 2006). Although these three methods can be used to fabricate the T-shaped microstructure or the inverted T-shaped cavity, they involve some complex processes, such as the bonding between independent layers, the precise control of exposure dosage and the etching of metal layers. Sameoto replaced SU-8 by a positive photoresist as the mold material, and the PDMS microstructure similar to a T shape was casted (Sameoto and Menon 2009). However, compared with SU-8, the range of the thickness of the positive photoresist is limited.

In this study, a novel method based on a sacrificial photoresist layer technique was introduced to fabricate a three-layer SU-8 mould with inverted T-shaped cavities. A positive photoresist is filled into the cavity formed by the first two-layer SU-8 to be a temporary substrate for the third SU-8 layer. The positive photoresist can be dissolved by the developer of SU-8. Thus, the sacrificial photoresist layer can be easily removed with the development of the third SU-8 layer, and no extra process is required. Recently, complex 3D microstructures have been used to study the contraction of multicellular tissues and the transduction of mechanical force by cells (Legant et al. 2009). To demonstrate this method, a PDMS sensing microchip with array of T-shaped cantilevers for studying the mechanics of cells was fabricated and tested.

2 Experimental details

2.1 Design of the SU-8 mould

The SU-8 mould is fabricated on a 2-inch silicon wafer (Fig. 1). The mould includes 12×16 array units. Each unit comprises two independent inverted T-shaped cavities, and dimensions of each unit are 750 μm×400 μm. The distance between these two cavities is 400 μm. Each cavity is made of three SU-8 layers. The first layer is used as the bottom of the cavity, and the other two layers compose the inverted T shape. The width and depth of the horizontal part of the inverted T-shaped cavity are 200 μm and 90 μm, and the width and depth of the vertical part are 130 μm and 110 μm, respectively.

2.2 Fabrication of the SU-8 mould

The fabrication steps of the three-layer SU-8 (2075, MicroChem Corp., Newton, USA) mould with inverted T-shaped cavities are schematically depicted in Fig. 2: (a) The first SU-8 layer was used to make the bottom of the cavity, and spin coated at 2,300 rpm for 30 s on a silicon wafer by a spinner (KW-4A, Institute of Microelectronics of Chinese Academy of Sciences, China). The SU-8 layer was soft-baked on a hot plate (AI-508 T, The 13th Research Institute of China Electronics Technology Group Corporation, China) at 60 °C for 10 min and then 85 °C for 60 min. The SU-8 layer was exposed to UV-light at 5.6 mW/cm^2 for 100 s by using a mask aligner (MA/BA6, SUSS MicroTec), and then post-baked at 85 °C for 1 min. (b) Under the same condition, the second SU-8 layer was coated and exposed to make the horizontal part. (c) The first two SU-8 layers were simultaneously developed in SU-8 developer (MicroChem Corp., Newton, USA). After rinsing with deionized water and drying with compressed nitrogen, the SU-8 layers were hard-baked at 85 °C for 30 min. (d) A positive photoresist (BP212, Beijing Institute of Chemical Reagents, China) was filled into the cavity formed by the first two SU-8 layers as the sacrificial material, and baked on the hot plate. (e) The excess BP212 was removed by using a precision lapping/polishing machine (UNIPOL-1502, MTI Corporation, USA). The lapping speed was 60~80 rpm, and the grit of the silicon carbide abrasive paper (Zhenjiang Fengmang Grinding Tools Co. Ltd, China) was CP3000. (f) The third SU-8 layer was spin coated at 1500 rpm for 30 s on the temporary BP212 substrate, and exposed at 5.6 mW/cm^2 for 130 s to make the vertical part of the inverted T-shaped cavity. (g) The third SU-8 layer was developed. Since BP212 can be dissolved by the developer of SU-8, the temporary BP212 substrate was removed at the same time. After development, SU-8 was hard-baked at 85 °C for 30 min. The cross section of a T-shaped cavity on the SU-8 mould was shown in Fig. 3a.

2.3 Replication of PDMS microchip

The PDMS microchip was fabricated by using the cast molding technique. Briefly, PDMS prepolymer (Sylgard 184, Dow Corning Corporation) and curing agent were mixed and stirred thoroughly. The mixture was poured on the SU-8 mold and degassed in a vacuum oven (DZ-2BC, Tianjin Taisite Instrument Co. Ltd, China) for 30 min. Then the mixture was heated to 85 °C and kept for 1 h in the oven. Finally, the PDMS microchip was peeled off from the SU-8 mould, and the cross section of a T-shaped cantilever on the microchip was shown in Fig. 3b.

2.4 Cell culture and microtissue seeding

C2C12 cell line was purchased from Cell Bank of Type Culture Collection of Chinese Academy of Sciences (Shanghai, China), which was cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10 % (v/v) Fetal Bovine Serum (FBS) at 37 °C with 5 % CO_2 using a cell incubator (Thermo Forma 371, Thermo Fisher Scientific). Adherent cells were dissociated with Trypsin-

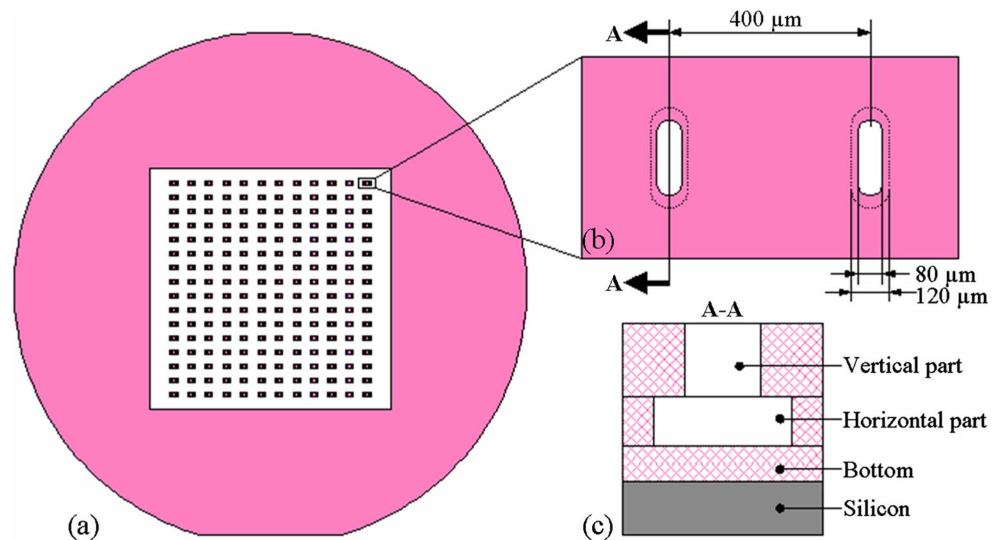

Fig. 1 Schematic diagram of the SU-8 mould. (a) Whole view of the SU-8 mould on a 2-inch silicon wafer; (b) Enlarged view of one unit; (c) Cross section of one inverted T-shaped cavity. Features are not drawn to scale

ethylenedinitriletetraacetic acid (EDTA) (0.25 %), and counted using a hemocytometer. The cell density was fixed at 105 cells/mL throughout the microtissue seeding.

The microtissue seeding was performed based on previously published procedures (Legant et al. 2012). Briefly, the PDMS microchip was first sterilized by 70 % ethanol and UV irradiation. Then, 2.5 mg/mL liquid neutralized collagen I from rat tail was added to the surface of the microchip. Cell-laden unpolymerized type I collagen was added to the microchip, and cells were centrifuged into the micropatterned wells on the microchip. Finally, excess collagen and cells were removed, and the collagen was polymerized at 37 °C. The cells were cultured for 7 days and imaged daily using an inverted microscope (Nikon TS100-F).

3 Results and discussion

3.1 Fabrication of BP212 temporary substrate

As stated above, BP212 was used to make a temporary substrate after the first two SU-8 layers were developed. Spin

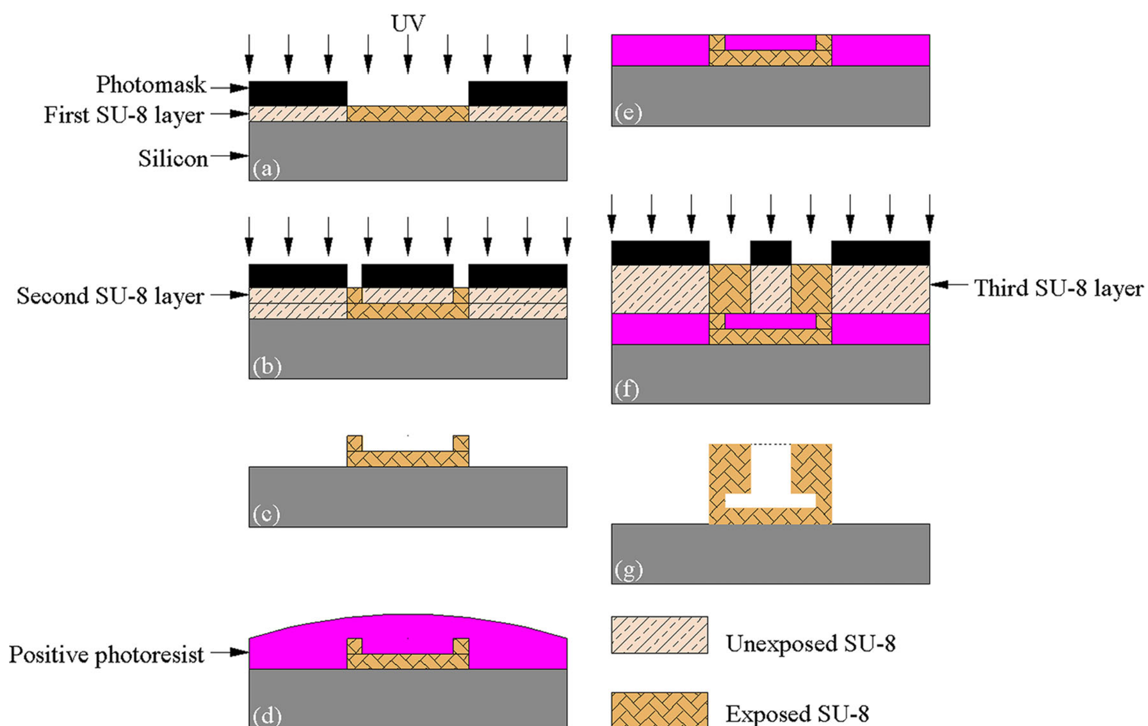

Fig. 2 Illustration of SU-8 mould fabrication. (**a**) Exposing of the first SU-8 layer; (**b**) Exposing of the second SU-8 layer; (**c**) Developing of the first two-layer SU-8; (**d**) Filling of positive photoresist; (**e**) Mechanical lapping; (**f**) Exposing of the third SU-8 layer; (**g**) Developing of the third SU-8 layer

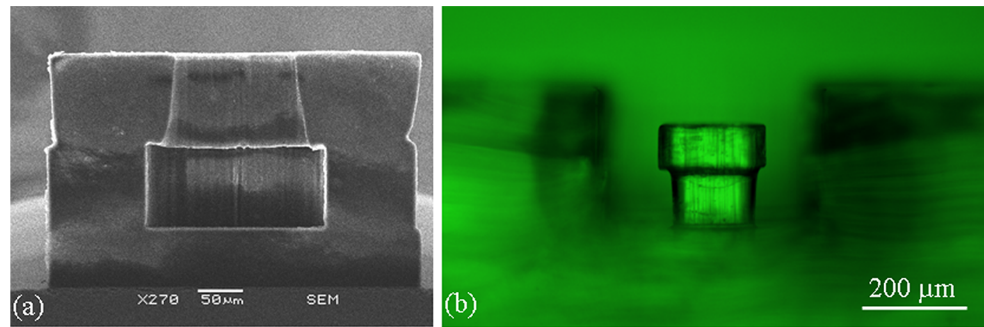

Fig. 3 (**a**) The cross section of the inverted T-shaped cavity; (**b**) The cross section of a T-shaped PDMS cantilever

coating is commonly used to form a photoresist layer, and the thickness of the layer can be precisely controlled. Therefore, the temporary substrate was initially fabricated by spin coating. However, the thickness of the BP212 layer made by each spin coating was only about several microns due to the low viscosity (31 mPa·s at 25 °C). To fill the cavity formed by the first two SU-8 layers, multiple coating and baking steps had to be performed, which is time-consuming and tedious. Hence, BP212 was directly dripped into the cavity, and the excess BP212 was removed by mechanical lapping.

BP212 was hardened by baking on the hot plate before lapping. It was observed that the removing of BP212 was very quick and hard to control if BP212 was not baked enough. On the other hand, thermal stress in BP212, SU-8 and the silicon substrate is induced from the thermal expansion coefficient mismatches, and it increases with the baking temperature. Thus, some cracks were observed in SU-8, and the silicon substrate even began to bend due to thermal stress when the baking temperature was higher than 85 °C. To address this, the baking temperature and time were optimized, and the hardness of the BP212 layer was measured by an ultra-light weight hardness tester (DMH-2LS, Matsuzawa Seiki Co. Ltd, Japan). The optimized condition was baking temperature of 65 °C and baking time of 5 h, and the Knoop hardness of the BP212 layer was 15.6 kg/mm^2, which gives a flat and uniform BP212 layer without any cracks. Besides, the circulating water system was applied during the lapping process. The water film formed significantly reduced the adhesion between BP212 and the sandpaper. The particles generated by lapping were quickly taken away by the circulating water, avoiding the clogging up of the sandpaper.

3.2 Demoulding of PDMS microchip

The inverted T-shaped cavity on the SU-8 mould makes the demoulding of the PDMS microchip much difficult. While PDMS is flexible, we found that the cured PDMS microstructure could be easily broken if it was peeled off directly from the SU-8 mould (Fig. 4). To address this, we developed a demoulding method by combining a few measures. First, the surface of the SU-8 mould was modified by using trimethylchlorosilane, which can reduce the adhesion between PDMS and SU-8 (Shao et al. 2012). The SU-8 mould and a Petri dish with a few drops of trimethylchlorosilane (Sinopharm Chemical Reagent Co. Ltd, China) were placed in a desiccator for 1 h. Second, the weight ratio of PDMS prepolymer and curing agent was changed from 10:1 to 15:1, which makes the cured PDMS more flexible. Third, the whole demoulding process was operated in anhydrous ethanol, where anhydrous ethanol could play a lubricating role and contribute to the demoulding (Tan et al. 2012).

Moreover, the lifetime of the SU-8 mould was tested. Two moulds were fabricated and used for multiple castings. After 10 castings, these two moulds still kept structural integrity, and no detachment of SU-8 was observed, which further demonstrates the feasibility of the demoulding method.

3.3 Deformation of microtissue

The PDMS microchip fabricated here can work as an experimental model for studying the mechanics of cells. The top of the T-shaped cantilever can provide a large attachment point for cells that are cultured in the chamber. As the cells

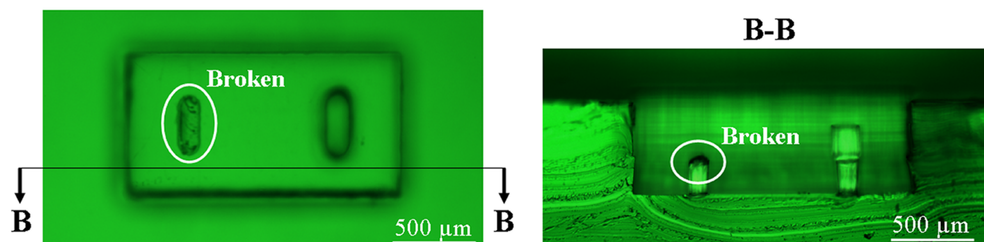

Fig. 4 The broken T-shaped PDMS cantilever

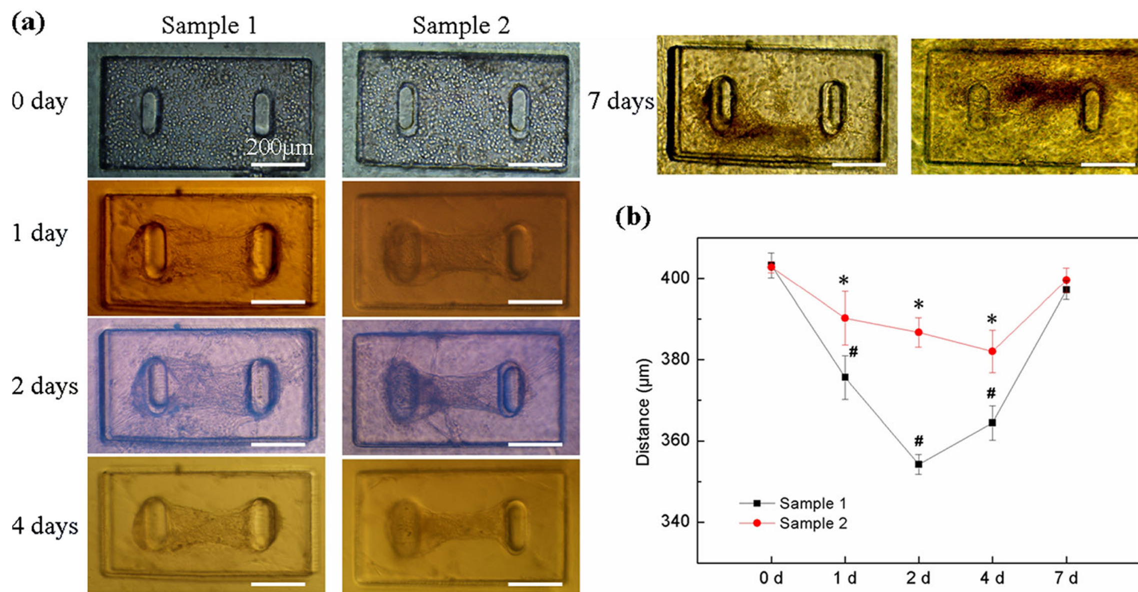

Fig. 5 Representative images of microtissue formation after 0–7 days culture (**a**) and changes of distances during microtissue contraction (**b**). For quantifying the deformation of microtissue, the distances (**d**) between two cantilevers were measured on day 0, 1, 2, 4, and 7. Data were shown as mean±standard deviation (SD). *P*-values less than 0.05 indicated the statistical significance after two-tailed student's *t*-Test. #, $P<0.05$, compared with control of sample 1; *, $P<0.05$, compared with control of Unit 2; student's *t*-Test; $n=3$

proliferate and grow between the two adjacent cantilevers, the physical force generated by cells (e.g., due to cell contraction) can cause the T-shaped cantilever to bend towards each other, which can be detected by a mechanical model correlating the cantilever's displacement.

To show the potential application of the PDMS microchip, C2C12 cells within type I collagen were added to the sensing unit and cultured for 7 days (Fig. 5). As shown in Fig. 5a, two random units were observed. Collagen matrices with cells anchored to the top of the T-shaped cantilever and began to spontaneously contract, and 3D microtissues were formed on day 1. To quantify the deformation of microtissues induced by the physical force generated by cells, the distance (d) between two cantilevers was measured on day 0, 1, 2, 4, and 7 (Fig. 5b). The minimum distances between two cantilevers for these two units were observed on day 2 and day 4, respectively. Then, the distance was increased and returned to the original level on day 7 due to the death of cells. The physical force can affect morphologic changes and regulate cellular phenotypes vice versa (Du et al. 2013), and it will be further quantified and investigated after measuring and calibrating the spring constant of the cantilever.

4 Conclusion

Based on a sacrificial photoresist technique, a method for fabricating a three-layer SU-8 mould with inverted T-shaped cavities was presented. By using the mould, a PDMS microchip with arrays of T-shaped cantilevers was made. The deformation of cantilevers induced by the physical force generated by C2C12 cells was measured. The PDMS microchip can play important roles in building 3D microtissues and monitoring the mechanical changes during the microtissue culture, and has many potential applications, such as the formation of 3D cardiac microtissues and drug screening. The fabrication method of the SU-8 mould developed here is simple and compatible with microfabrication techniques, and thus can be extended for constructing varieties of complex SU-8 microstructures at low cost.

Acknowledgments This work was supported by the National Natural Science Foundation of China (51075056, 81301040, 91023046, 11372243), Program for New Century Excellent Talents in University of China (NCET-10-0284, NCET-12-0437), International Science & Technology Cooperation Program of China (2013DFG02930, S2013GR0241), China Young 1000-Talent Program, Dalian Foundation of Science and Technology (2012J21DW002), and Program for Youth Science and Technology Star of Shaanxi Province (2014KJXX-76).

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