A simple method for fabrication of microarrays and microfluidic device using PDMS stamp

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ABSTRACT

We propose a simple approach to fabricate a series of microwell arrays using the conventional PDMS (Polydimethylsiloxane) stamp approach to study the cell-to-cell adhesion of Human Bone Marrow Mesenchymal Stem Cells (BM-MSCs). This approach can be applied to fabricate a microfluidic channel with glass-PDMS-glass configuration for cytologic diagnosis. To replicate the microwell arrays and the microfluidic channel, an SU-8 master mold is created, and then a PDMS stamp replica is transferred to a glass substrate. For the pattern transfer from the PDMS stamp to the glass substrate, the surface of the PDMS stamp is treated by oxygen plasma and bonded on the glass substrate. By ripping the PDMS stamp by means of applying a mechanical force, the PDMS patterns are formed on the glass substrate. For the cell-to-cell interaction studies, 4 different types of PDMS arrays are created to vary cell-to-cell contact length. For the cytologic diagnosis, the microchannel is designed, that allows for the enrichment and temporary immobilization of the cells.

Keywords: microwell array, glass-PDMS-glass fabrication, pattern transfer

1 INTRODUCTION

Patterning of microarrays on a surface has become a very attractive tool for sample detection and quantification purposes. [1] There has been a growing interest in applying this technique for various cell studies. Thus, it is important to develop efficient fabrication methods for the microarray which can offer lower costs and simpler production methods for various biological applications[1-3]. Due to the fact that PDMS can be easily fabricated by using soft-lithography techniques, it was widely used for this experiment. [4-5].

In the early stages of cell-to-cell interaction studies, Petri dishes were used to form cell-to-cell contact [5]. However, the ability of observe the cells that grew in the dish was limited due to the low desnity of each pair of cells. Moreover, under the conventional cell culture conditions, it is hard to manipulate the cell formation. To regulate the cell-to-cell contact with various shapes, densities, and contact of cells, micropatterns can be printed onto a cell culture substrate using the PDMS stamp inked by adhesive molecules [6-8]. This technology can offer a cell culture environment with well-controlled sizes, shapes, and positions on a substrate, thus providing a useful tool for cell studies. As an alternative, an agarose pattern on a glass substrate can be fabricated using the PDMS stamp method [9]. The agarose is perfused under the PDMS stamp attached on the glass. Thus, the region sealed against glass remian free of agarose. After the agarose cures, the PDMS stamp is removed, leaving behind a well defined geometry of agarose patterns. Currently, most microarrays are fabricated by the contact printing technique using a PDMS stamp for a controlled cell culture environment. Despite its huge potential, the contact printing method should be carefully controlled to avoid nonuniform patterns, as deformation can occur when handling the stamp. In the printing technology, the microarrays are typically influenced by the surface tension and viscosity of samples [10].

We describe a simple microwell array fabrication technique to isolate the effects of cell-to-cell contact by transferring PDMS patterns on a glass substrate. To overcome the technical challenges in the contact printing technique, a simple and robust fabrication method is demonstrated to study the role of intercellular adhesion forces in myogenic differentiation of stem cells and the molecular pathways governing this process. The extent of the cell-to-cell adhesion can then be examined by immunostaining various cadherin molecules which control BM-MSCs differentiation towards smooth muscle lineage. In particular, we study levels of Cadherin-11 (Cad-11) and its effect on smooth muscle genes α-SMA (Alpha- Smooth Muscle Actin), CNN-1 (Calponin) and MYH11 (Myosin-Heavy Chain). We expect that this tool can be further extended to 3 or 4 neighboring cells in micropatterns, thereby establishing Cadherin-11 as a master regulator of BM-MSCs to smooth muscle differentiation. Furthermore, we demonstrate a very useful method for a microfluidic device with glass-PDMS-glass configuration for the effectiveness of the proposed method. Thus, the proposed method can be used to generate the microarrays and

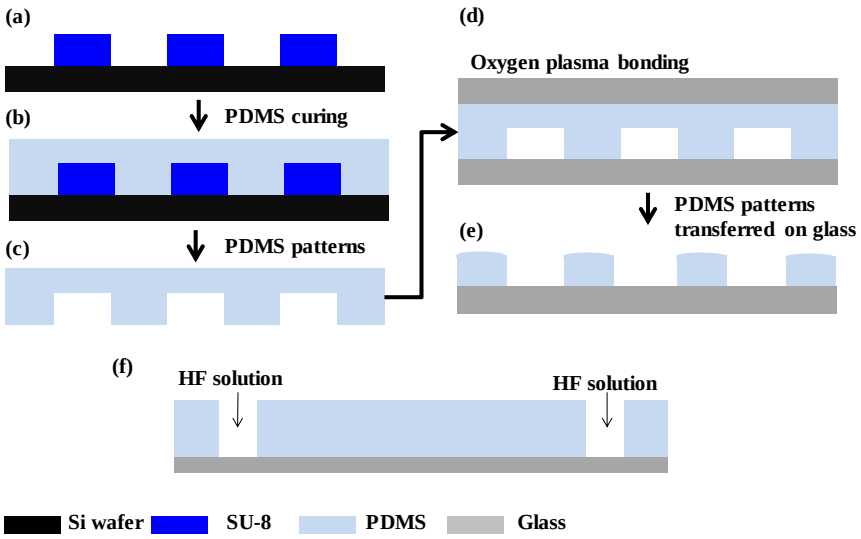

Fig. 1 Working principle for the PDMS microwell array on the glass slide. (a) Patterning of SU-8 photoresist by photolithography. (b) Replication of the PDMS stamp. (c) Peeling off the PDMS stamp from the master mold. (d) Bonding the PDMS stamp to two glass slides. (e) Transfering the patterns of the PDMS stamp to the bottom glass by applying a force to the top glass. (f) Formation of through hole by HF solution for the inlets and outlets.

microfluidic channels which provide an attractive new approach over the conventional methods.

2 METHODS/EXPERIMENT

2.1 Microarrays and Microfluidic device design

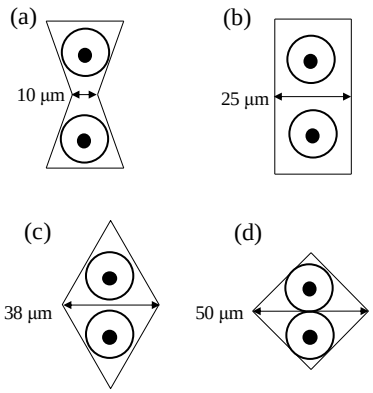

Fig. 2 4 different types of micropatterns used to trap two cells for cell-to-cell contact: (a) Bowtie (b) Rectangle (c) Rhombus and (d) Wide-rhombus.

The size of each PDMS stamp was 5 mm × 5 mm and the array of each pattern has 10 μm distance between patterns. **Fig. 1** shows the configuration of 20 μm thick PDMS pattern fabrication on the slide glass for cell-to-cell adhesion studies in the microwell array. By conventional soft-lithography processes, 4 different types of PDMS patterns (e.g., rectangle, bowtie, wide-rhombus, and rhombus) were replicated from a master mold as shown in **Fig. 2**. The density of the patterns and the contact surface area between the patterns and the glass were listed in Table 1.

To fabricate the microfluidic channel based on the proposed method, a prepolymer and curing agent were mixed at a 20:1 ratio. The microfluidic device was fabricated with the channel dimensions as follows: 6.1 cm in length, 5 mm in width and 100 μm in height.

Table 1 PDMS patterns for cell-to-cell adhesion

Shape	Rectangle	Bowtie	Wide Rhombus	Rhombus
Density of pattern (#/mm²)	937.11	797.20	641.46	946.84
Contact area of PDMS (%)	59.91	50.18	41.43	40.82

2.2 Fabrication

In order to create the SU-8 master mold, a series of flim photomasks were designed using AutoCAD commerical software (Autodesk, USA), and then printed on transparencies (CAD/Art Service Inc., Bandon, OR, USA). The PDMS patterns were fabricated by using conventional soft-lithography techniques. In order to make a master mold, negative photoresist (SU-8 2015, Micro-Chem Corp, Newton, MA, USA) was used, and then spin coated with target thickness (e.g., 20 μm) at 2000 rpm on a cleaned wafer using a spin processer (WS-650Mz NPP from Laurell Technologies, North Wales, PA, USA). Before the coating process, the silicon wafer was submerged into BHF (buffered hydrofluoric acid) at room temperature for 5 min to remove a thin oxide layer that can make a weak adhesion

between the SU-8 and the surface of the wafer. Afterward, it was cleaned with acetone, followed by methanol. It was then rinsed by DI water and blown dry with filtered nitrogen gas. After the spin coating process, a soft bake process was conducted at 95 ℃ for 4 min. After UV exposure through the film photomask and subsequent post exposure baking process was conducted at 95 ℃ for 5 min, the wafer was developed in SU-8 developer (Microchem, USA) and washed with isopropyl alcohol to completely remove the developer. Using a gentle stream of nitrogen gas, the wafer with SU-8 patterns was dried. The measured thickness of SU-8 pattern was 20 ± 2 μm.

The PDMS patterns were fabricated from PDMS prepolymer and curing agent (Sylgard 184, Dow Corning Co., Midland, MI). The two materials were thoroughly mixed at a ratio of 20 : 1 (wt/wt). The mixed PDMS was degassed in a vacuum chamber to remove any air-bubbles for 20 min. The SU-8 master mold was silanized using hexamethyldisilazane (Sigma Aldrich, Saint Louis, MO, USA) in a vacuum chamber for 3 hours to easily peel off the PDMS from the SU-8 master mold. To mold the PDMS against the master mold, it was carefully poured onto the SU-8 master mold and cured at 65℃ for 30 min. The PDMS replica was peeled off and bonded irreversibly to a glass by exposing it to O_2 plasma. The flat surface of PDMS was bonded to the glass, and then the surface of PDMS with the patterns was sandwiched with another glass. Finally, the glass bonded to the PDMS patterns was released from the patterns.

3 RESULT/DISCUSSION

As shown in **Fig. 3**, the patterns were successfully fabricated on the glass by causing the crack. The patterns have 20 μm thick and 10 μm gap among the patterns. The thickness was enough to trap the cells into the wells. The force was applied at the edge of the glass in order to locally initiate the crack. The crack started from a mechanically weak corner of the PDMS so that the different shapes can be transferred from the PDMS to the glass.

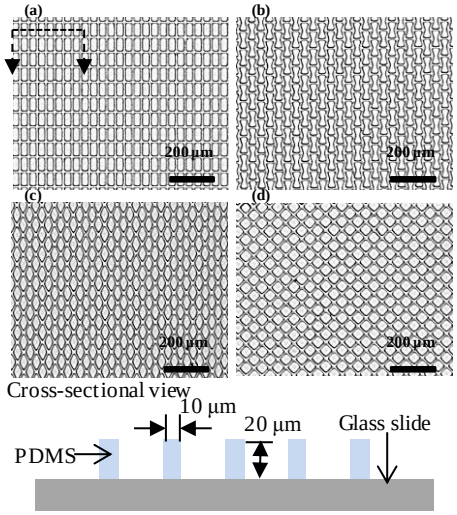

Fig. 3 Photographs of PDMS patterns on the glass substrate. (a) Rectangle. (b) Bowtie. (c) Wide Rhombus. (d) Rhombus shape.

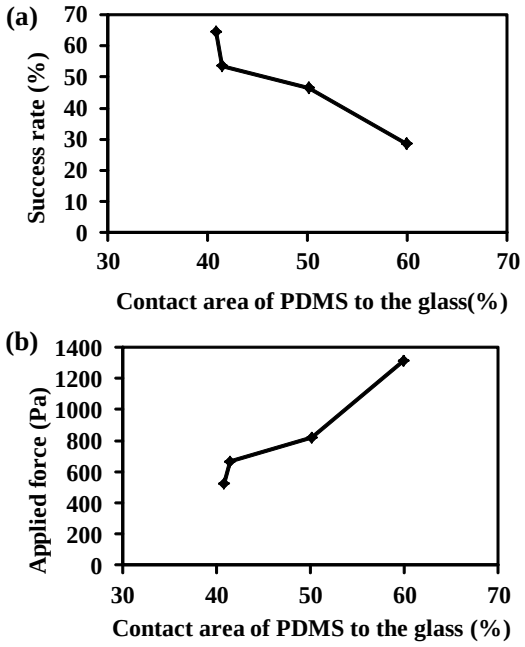

Fig. 4 (a) Success rate of patterns transferred to the glass according to the contact area of PDMS patterns. (b) Applied force to the top glass to create the crack on the patterns.

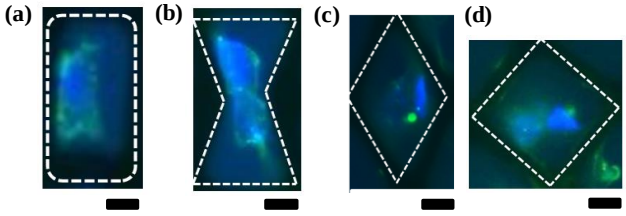

Fig. 5 Fluorescent images showing cell-to-cell adhesion in each pattern. The trapped cells in the patterns were allowed to attach for 48 hours. The samples were counterstained with DAPI for nuclei. (a) Rectangle. (b) Bowtie. (c) Wide Rhombus. (d) Rhombus shape. The scale bar is 10 μm.

After the patterns are bonded, it is simply torn from the bottom glass using the home-made equipment to release the top glass. Only patterns tear at the locations where the patterns are bonded to the bottom glass. It is best to tear the PDMS patterns, applying torque force at the position 50 mm away from the edge of the glass in order to locally initiate the crack. In the **Fig. 4 (a)**, the percentages show the success rate of patterns transferred to the glass for each

condition given in Table 1. It can be seen that the patterns with smaller contact area are more easily created since the crack resistance is low so that the crack starts more uniformly along the edge of patterns at mechanically instable point. However, with increasing contact area, the success rate will gradually decrease due to the nonuniformity of the crack. We investigated the maximum force applied at the top slide glass to tear the pattern by our

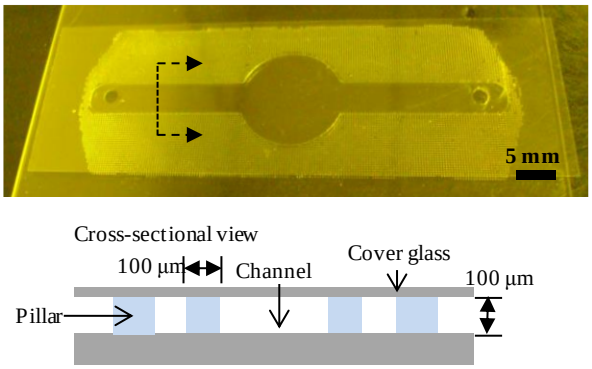

Fig. 6 Photograph of the microfluidic channel with glass-PDMS-glass configuration. The transferred channel was bonded to the cover glass with the inlet and outlet that are formed by the etching using the HF solution.

personal equipment. The mechanical force was applied at the position of glass 50 mm away from the edge of the PDMS patterns. The measured moment of force was ranged from 0.5 to 1.31 kPa as shown in **Fig. 4 (b)**.

As a demonstration of the effectiveness of this method, the cell-to-cell adhesion was performed on the each pattern. Cells were loaded into microwells so that one pair of cells was cultured within each microwell, resulting in contacting a single neighboring cell as shown in Fig. 5.

To demostrate this proposed technique for glass-PDMS-glass device, a simple microfluidic device was fabricated as shown in **Fig. 6**. This device consisted of a main channel and a circular region to allow cells to spread out. For the inlet and outlet, high concentrated HF solution of 48% was used to etch the holes on the cover glass for 20 minutes. The cover glass with the through holes was bonded onto the PDMS patterns for the microfluidic channel by an oxygen plasma treatment.

4 CONCLUSION

We have demonstrated the simple and low-cost fabrication method of microwell array on a glass slide using the conventional photolithography for cell-to-cell adhesion-mediated differentiation to smooth muscle cells. 4 different types of patterns were created on the glass substrate and the pattern transfer rate and applied force was investigated as the function of contact area of PDMS to the glass. We believe that the isolated cell pairing into the microwells and the separate analyses of the cell patterns that were cultured, allowed for a more efficient analysis from a small quantity of cells, which is highly important when using rare samples. In addition, the microfluidic channel with glass-PDMS-glass configuration was fabricated based on the proposed method, which allowed for the enrichment and temporary immobilization of cells.

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