

# Patterned PDMS based cell array system: a novel method for fast cell array fabrication

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**Abstract** Cell-cell interaction is important in numerous biological processes, including cell growth, cell differentiation and migration. The ability to generate pre-determined cell patterns or cell arrays on a study surface is crucial for cell-cell interaction studies. In this paper, we developed a method for fast cell array fabrication using laser sintering and the hydrophobicity of PDMS films. This approach can be easily adopted and is cost-effective. Hydrophobic PDMS films were fabricated into polymeric chips containing hundreds of microwells. The films were then transferred onto tissue culture surfaces to separate cells in the formation of cell arrays (Patterned PDMS based cell array system, PCAS). We used NIH/3T3 fibroblast cells to demonstrate the feasibility of PCAS. The success of fast fabrication of patterned cell arrays was obtained using different initial cell seeding densities. We also used poorly adherent PC-12 cells to demonstrate the cell-cell communication. Results showed that the method is very useful for studying topics such as cell-cell interaction, cell-substrate interaction or cell migration.

**Keywords** Microarray · Cell array · Hydrophobic PDMS · Cell-cell interaction

## 1 Introduction

Cell-cell interaction is important in numerous biological processes, including cell growth (Bhatia et al. 1998), cell

differentiation and formation of functional tissues *in vitro* (Bhatia et al. 1998; Langer and Vacanti 1993). The ability to generate pre-determined cell patterns on the substrate surface is a key step for studying tissue formation and cellular function *in vitro*, which are usually regulated by diverse biological factors including cell–cell communication, cell–matrix interactions, and soluble factors (Attucks and Katula 2001; Campbell et al. 2006; Heasley 2001; Javazon et al. 2001; Quehenberger 2005). Patterning cells on a specific area with defined shapes are also necessary for investigating the function of cell interaction (Campbell et al. 2006; Rosenthal et al. 2007) and the migration of cell in substrate (Nakanishi et al. 2007; Onuki-Nagasaki et al. 2008).

Numerous methods have been developed to pattern cells, such as photolithography (Iwanaga et al. 2005), micro-contact printing ( $\mu$ CP) (Wang et al. 2007; Bernard et al. 2000; Kane et al. 1999; Singhvi et al. 1994) and ink-jet printing (Roth et al. 2004). Typically, photolithography technology involves a light-guided crosslinking process and has limitations on nonplanar surfaces. In  $\mu$ CP, a stamp containing defined features is fabricated by soft lithography and molding of the elastomeric stamp. It is used to imprint surfaces with specific extracellular matrix protein that mediate cell attachment. This technique is attractive because of its high accuracy and ease of duplication. Ink-jet printing can deposit protein by robotic machine that reduces the cost and time of fabrication (Roda et al. 2000). However, both of these methods require specialized equipment, and the surfaces are often modified with supporting extracellular matrix protein for cell attachment.

Polydimethylsiloxane (PDMS) is widely used for the fabrication of microfluidic devices (McDonald et al. 2000) and elastomeric stamp for  $\mu$ CP because of its good biocompatibility (Lee et al. 2004; Park et al. 1999), low

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toxicity and low cost. Due to the low surface energy and hydrophobicity, PDMS surfaces inhibit cell adhesion (Patriito et al. 2007). However, a good adhesion is obtained between PDMS and polished surfaces like tissue culture polystyrene plate (TCPS) and glass (Lotters et al. 1997). Therefore, the elastomeric and adhesive properties make the PDMS thin film as a potential material for the fabrication of patterned microarrays.

In this paper, we developed a new strategy by combining laser sintering with the hydrophobicity of PDMS films for a faster fabrication of thin film microarrays. Laser sintering combined with computer drawing software can significantly decrease the cost in the fabrication procedure when compared to the lithography process for  $\mu$ CP. Laser sintering method has the advantages of automatic robotic fabrication and precision within the micrometer scale. Hydrophobic PDMS film could be fabricated into polymeric chip containing hundreds of microwells by a laser sintering machine. The PDMS film was then transferred and bound to a culture surface to separate cells in the formation of cell arrays (Patterned PDMS based cell array system, PCAS). We used the adherent cell NIH/3T3 fibroblast and poorly adherent PC12 cells to demonstrate the feasibility of PCAS. We also described the method for fast fabrication of PCAS and its advantages such as substrate independent, controllable patterns and suitable for large-scale patterning.

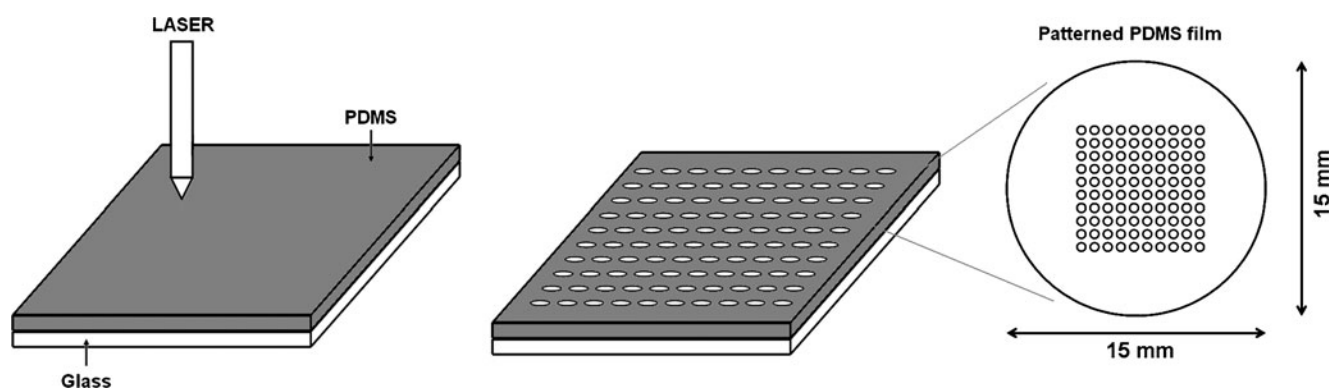
## 2 Materials and methods

The patterned PDMS based cell array system (PCAS) consists of microfabricated polymer chip containing hundreds of microwells and cell culturing plates (Fig. 1). Cell array is easily formed with this polymeric chip. Figure 2 shows the schematic representation of the process for fabricating cell array with adherent NIH/3T3

cells. Briefly, patterned PDMS films were attached to the 24-well culture plates. After seeding fibroblast, the cells were allowed to attach onto the culture plate freely for several hours. Due to the hydrophobicity of PDMS, fibroblast cells only adhere to the uncovered area. After removing the PDMS film, cell arrays were formed. These microwells can be designed to fit cell clusters of different sizes according to the need of each experiment. PCAS requires no external equipment or chemical modification for cell patterning, making this technology easily adoptable. The patterned PDMS films are easily bound to the surface of culture environment, like TCPS and glass, and cells can then be pipetted onto the surface of the chip. Cells fall into the microwells created by the PDMS films and adhere to the culture plate after a few hours. After the cell adhesion, the PDMS films can be removed and well-defined cell arrays are obtained, as shown in Fig. 3. In the following section, we will demonstrate the method more precisely.

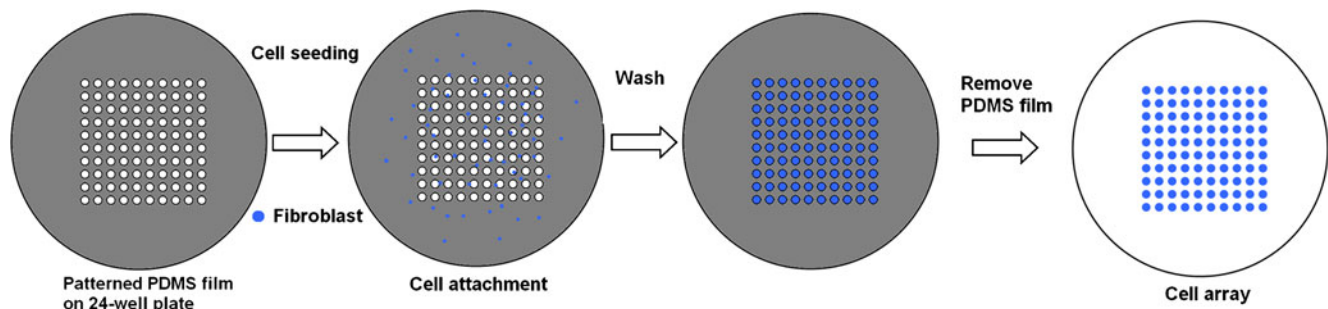
### 2.1 Preparation of patterned PDMS arrays

Patterned PDMS films were fabricated following a previously established method (Hsieh and Huang 2009). In brief, PDMS oligomer (Dow Corning, Midland, MI, USA) was mixed with crosslinking agent in a weight ratio of 10:1. The mixture was stirred until uniform, then degassed by vacuum pump. The resulting PDMS solution was spin-coated on the glass substrate with a thickness of 100  $\mu$ m. These glass plates were placed in a 65°C oven for 12 h for hardening. Patterns of microwells with different sizes (ranging from 100 to 500  $\mu$ m) and suitable center to center space were designed using AutoCAD (Autodesk, San Rafael, CA, USA) software. The designed pattern in AutoCAD was imported into a Windows (Microsoft) based computer which controlled a CO<sub>2</sub> laser (Laserpro Venus®, GCC, Tamworth, UK). After laser sintering, the patterned PDMS



**Fig. 1** Schematic representation of the laser sintering process for fabricating the patterned PDMS film. The patterned PDMS films were 15 mm in diameter for fitting inside the 24-well culture plates. Arrays with different diameter and center to center spacing were designed

according to the requirement of the experiment. The patterned films were cleaned with sonication using 70% ethanol solution and stored in clean ethanol solution



**Fig. 2** Schematic representation of the process for fabricating cell array with adherent NIH/3T3 cells. Patterned PDMS films are attached to the 24-well culture plates. The air bubbles trapped in the patterned wells are removed by washing with ethanol and PBS. After seeding

fibroblast, the cells are allowed to attach onto the culture plate freely for several hours. Due to the hydrophobicity of PDMS, fibroblast cells only adhere to the uncovered area. After removing the PDMS film, cell arrays are formed

films were removed from the substrate and washed by sonication in 70% ethanol. The films were stored in 70% ethanol prior to usage.

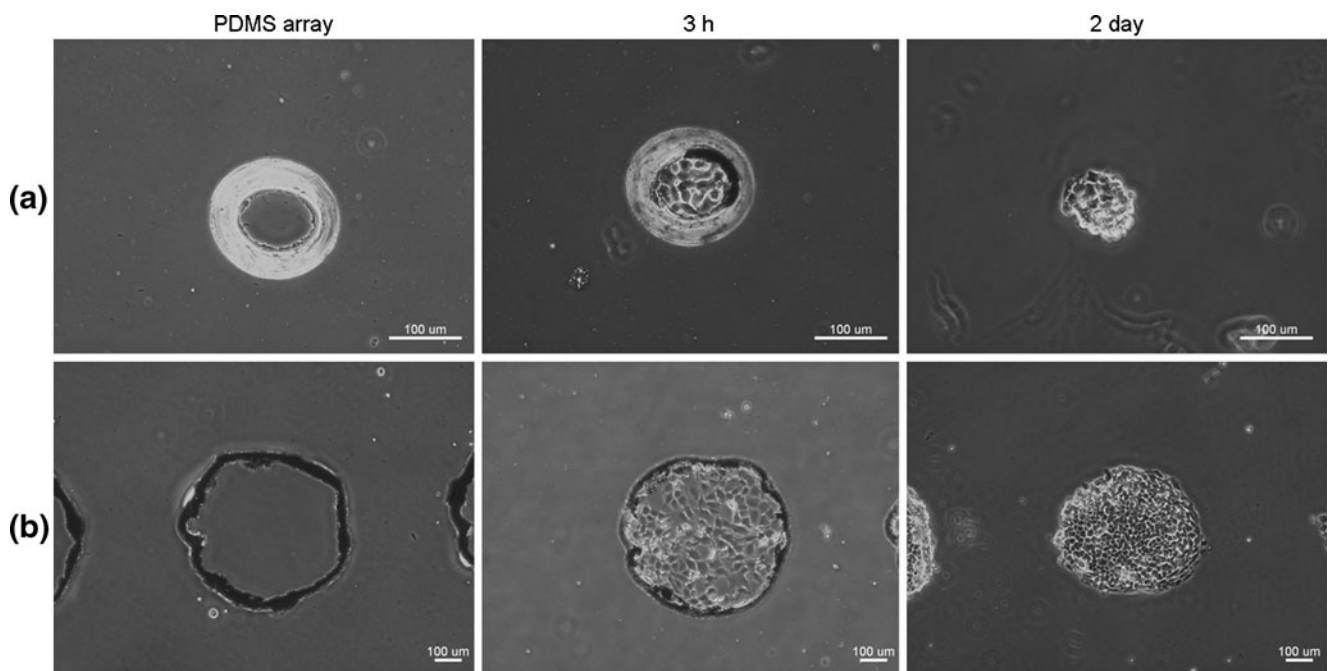
## 2.2 The patterned PDMS based cell array system (PCAS) operation

The patterned PDMS films were transferred from storage to sterile culture dishes and dried in a laminar flow hood. After drying, the films were attached to culture plates or cover glasses using forceps. Few drops of 70% ethanol were gently pipetted onto the films to reduce the air bubbles

that were trapped in the wells. The culture plates were incubated with culture medium at 37°C in a humidified 5% CO<sub>2</sub> atmosphere followed by two times of PBS washing.

## 2.3 Cell culture

The patterned arrays were cultured with NIH/3T3 fibroblast and PC12 cells. NIH/3T3 fibroblast cells were maintained in Dulbecco's Modified Eagle Medium (DMEM, Invitrogen, Carlsbad, CA, USA) supplemented with 10% (v/v) fetal bovine serum (FBS, Invitrogen) and 1% (v/v) penicillin/streptomycin (Sigma-Aldrich, St. Louis, MO, USA). PC12



**Fig. 3** Optical images of patterned PDMS films and the fabricated cell arrays. Row (a) and (b) show the arrays with diameter of 100 μm and 350 μm respectively. The center to center spacing for adjacent cell colonies is 500 μm in both cases. The left column shows the structures of PDMS films attached onto the culture plate. The middle column

shows cells attached to the patterned wells on culture plate. Few cells attach to the surface of PDMS films, and most cells attach onto the patterned wells. The right column shows the cell morphologies on the patterned wells after 2 days of culture. Cells grow and become confluent on patterned wells. Scale bars in these images are 100 μm

cells were maintained in RPMI medium 1640 (Invitrogen) supplemented with 10% (v/v) horse serum (HS, Invitrogen), 5% FBS, 1% (v/v) penicillin/streptomycin, and 1 mM sodium pyruvate (Sigma-Aldrich). The differentiation medium for PC12 cells was low serum RPMI medium 1640 which contained 1% (v/v) HS, 0.5% FBS, 1% penicillin/streptomycin, 1 mM sodium pyruvate, and NGF (5 ng/ml).

### 2.3.1 Fabrication of PCAS for adherent cell

The PDMS films were patterned into  $10 \times 10$  arrays with a diameter of 100  $\mu\text{m}$  or 350  $\mu\text{m}$ , and placed onto 24-well culture plates. The spacing distance (center to center) between two adjacent arrays was 500  $\mu\text{m}$ . NIH/3T3 fibroblasts were trypsinized and seeded at a density of  $5 \times 10^2$  cells/ $\text{mm}^2$ . The culture plates were incubated for 3 h for cell attachment. Then the medium was drained and washed with PBS three times to remove any non-adherent cells. The patterned PDMS films were removed from the culture plates after 2 days of incubation.

### 2.3.2 Fabrication of PCAS for poorly adherent cell

For poorly adherent cell, 1 ml polylysine (50  $\mu\text{g}/\text{ml}$ ) were added to the 24-well culture plates containing patterned PDMS films. The films contained a  $10 \times 10$  array, as described in 2.3.1, except the diameters were changed to 150  $\mu\text{m}$  and 350  $\mu\text{m}$  respectively. The culture plates were incubated at  $4^\circ\text{C}$  overnight to allow the physical absorption of polylysine. Before cell seeding, the polylysine solution was removed and the culture wells were washed with PBS. Two different cell seeding procedures were performed, as shown schematically in Fig. 4: procedure A where the

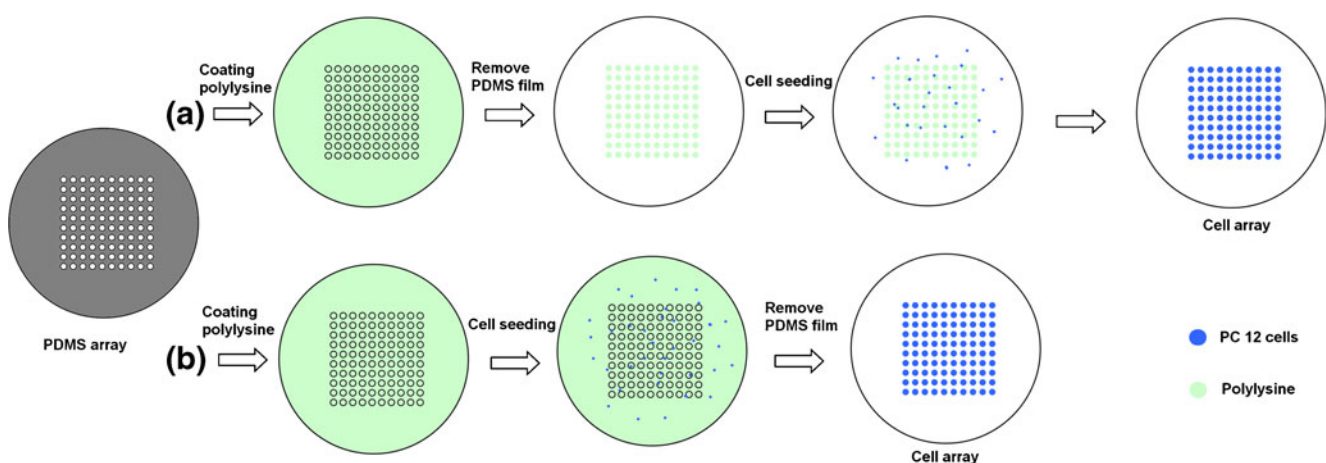
patterned PDMS films were removed before cell seeding, and procedure B where cells were seeded onto the culture wells directly and the patterned PDMS films were removed at the time of washing after 3 h of incubation. PC12 cells were seeded at a density of  $4.2 \times 10^2$  cells/ $\text{mm}^2$ .

### 2.4 The effect of cell seeding density for fast fabrication of PCAS

The patterned arrays were seeded with NIH/3T3 fibroblasts at densities of  $5 \times 10^1$ ,  $5 \times 10^2$ , and  $5 \times 10^3$  cells/ $\text{mm}^2$  in 24-well culture plates. The culture plates were incubated for 3 h for cell attachment. Then the medium was drained and washed with PBS three times to remove any non-adherent cells. After 1 day of incubation, the cells were washed with PBS and fixed with 4% paraformaldehyde. The cells were stained with Hoechst 33342 (Invitrogen) for nucleus and cells in each well was recorded by fluorescent microscope. The numbers of cells were analyzed by image J.

### 2.5 The differentiations of PC12 cells on PCAS

A  $10 \times 10$  PDMS array with a diameter of 250  $\mu\text{m}$  and center to center spacing of 400  $\mu\text{m}$  was used for PC12 cells differentiation experiments. The patterned plate was incubated with polylysine at  $4^\circ\text{C}$  overnight. PDMS films were removed before seeding. PC12 cells were seeded at a density of  $4.2 \times 10^2$  cells/ $\text{mm}^2$  to each well. The PC12 cells were maintained in RPMI 1640 medium for cell attachment. After 1 day of incubation, the medium was replaced with low serum RPMI 1640 medium for cell differentiation. The medium was renewed every 2 days.



**Fig. 4** Schematic representation of the process for fabricating cell array with poorly adherent PC12 cells. Patterned PDMS films are attached to the 24-well culture plate. There are two different protocols. In process (a), after polylysine coating, the culture plate is washed by PBS and then the PDMS films are removed. The seeded cells attach

only to the area of polylysine-coated region but not the non-coated region. In process (b), the cells are seeded on the polylysine-coated PDMS films directly. Cells attach onto the patterned wells and PDMS film. After cell attachment, we remove the PDMS films and the cell arrays are formed



## 2.6 The application of PCAS in a cell migration study

A 6×6 PDMS array with diameter of 250  $\mu\text{m}$  and center to center spacing of 600  $\mu\text{m}$  was used for this cell migration study. NIH/3T3 fibroblast cells were seeded at cell density of  $10^3$  cells/ $\text{mm}^2$ . After 1 day of incubation, the PDMS films were removed and 20  $\mu\text{M}$  Y27632 dihydrochloride monohydrate (Y27632, Sigma-Aldrich), an inhibitor of ROCK (Rho-associated kinase) (Kuwahara et al. 1999) was added. The medium with 0.1 % DMSO added was used as control. Time lapse images were recorded for identifying the morphology of cell migration. The PCAS were immunofluorescent stained: the actin was dyed with Phalloidin–Tetramethylrhodamine B isothiocyanate (Phalloidin-TRITC, Sigma-Aldrich) and the nucleuses were dyed with Hoechst 33342. Specifically, PCAS were fixed with 4% paraformaldehyde in PBS, blocked in 50 mM BSA in PBS, permeabilized in 0.1% Triton X-100 in PBS and stained with phalloidin-Tritc and Hoechst 33342. Between each incubation step, cells were washed three times in PBS.

## 3 Results

A Laserpro Venus carbondioxyde laser was used to sinter the microwells of all PDMS arrays. The patterned PDMS films were removed from the substrate and transferred to the sonicator containing 70 % ethanol solution to clean the residue occurred in the laser sintering process. The cleaned patterned PDMS films were transferred to the culture plate and immersed with 70% ethanol solution until use. The structures of the two types of PDMS films were shown in Fig. 3. Different shapes of PDMS films can be designed in the computer sketching program, and automatically engraving by laser machine. The diameters of the microwells were measured to be within a variability of 5%.

### 3.1 Formation of adherent NIH/3T3 fibroblast PCAS

NIH/3T3 fibroblast cells were seeded into the PDMS microarrays and the cells were allowed to attach for 3 h and then cultured for 2 days after a fresh exchange of medium. The patterned PDMS films were removed from the culture plate after 2 days of incubation. Figure 3 showed the structures of the PCAS. Row A and B represented the 10×10 PDMS arrays with a diameter of 100  $\mu\text{m}$  and 350  $\mu\text{m}$ , both with a center to center spacing of 500  $\mu\text{m}$ . The left column showed the states of PDMS films attaching to the culture plate. The middle column showed the morphologies of PCAS after 3 h of incubation. The right column showed the morphology of cell cluster after 2 days of cultivation, where the cells had became confluent on patterned microwells.

## 3.2 Formation of poorly adherent PC12 PCAS

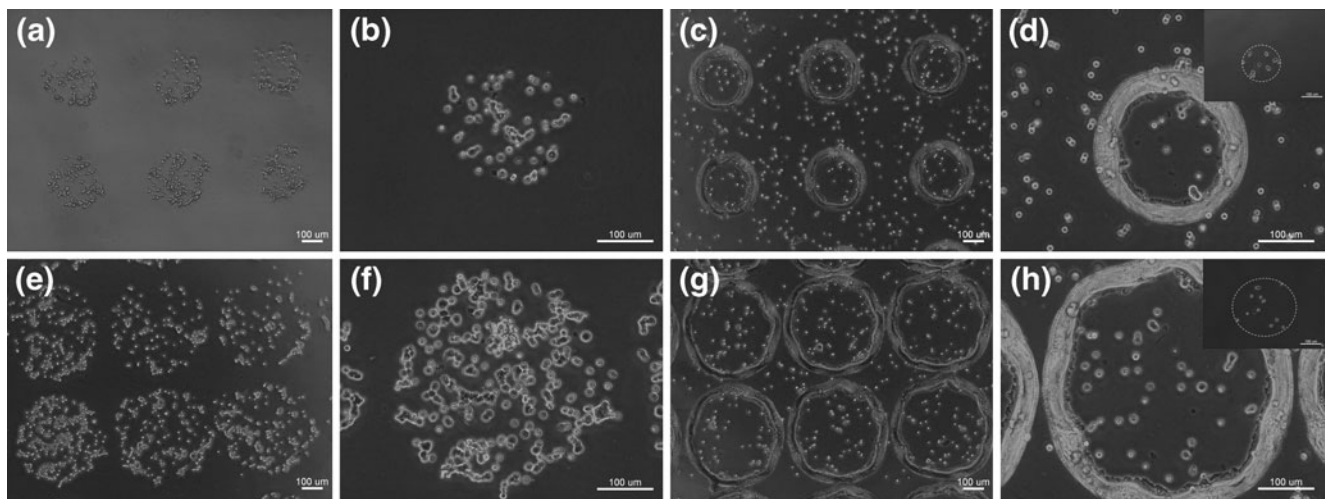
Before cell seeding, the PDMS microarrays were coated with polylysine at 4°C overnight and then washed with PBS. Two processes were tested in the cell attachment experiments. In procedure A, the patterned PDMS films were removed from the culture wells before PC12 cell seeding (Fig. 4(a)). The unattached cells were washed away and the medium was renewed after 3 h of incubation. The PCAS were approximately 150  $\mu\text{m}$  (Fig. 5(a) and (b)) and 350  $\mu\text{m}$  (Fig. 5(e) and (f)) in diameter with a center to center spacing of 500  $\mu\text{m}$ . The PC12 cells were confined to the polylysine coating areas and did not adhere to the area without polylysine coating. In procedure B, the patterned PDMS films were not removed after polylysine coating (Fig. 4(b)). The PC12 cells were seeded and allowed to attach to the PDMS films and polylysine coated area (Fig. 5 (c), (d), (g), and (h)). After 3 h of incubation, the medium and unattached cells were removed and washed with PBS. Then PDMS films were removed and the PCAS was formed (Fig. 5(d) and (h) insert).

### 3.3 The effect of cell seeding density for fast fabrication of PCAS

PDMS films with an approximately diameter of 150  $\mu\text{m}$  and 350  $\mu\text{m}$  and a center to center spacing of 500  $\mu\text{m}$  were seeded with  $5 \times 10^1$ ,  $5 \times 10^2$ , and  $5 \times 10^3$  cells/ $\text{mm}^2$ . The medium was renewed after 3 h of incubation. After 1 day of incubation, the cells were fixed with 4% paraformaldehyde solution and the nuclei were stained with Hoechst 33342 for counting. The images of PCAS were shown in Fig. 6. The cell arrays with 150  $\mu\text{m}$  diameter and cell seeding density of  $5 \times 10^1$ ,  $5 \times 10^2$ , and  $5 \times 10^3$  cells/ $\text{mm}^2$  were shown in Fig. 6(a), (b), and (c). The PCAS of 350  $\mu\text{m}$  diameter and cell seeding density of  $5 \times 10^1$ ,  $5 \times 10^2$ , and  $5 \times 10^3$  cells/ $\text{mm}^2$  were shown in Fig. 6(d), (e), and (f).

### 3.4 The differentiation of PC12 cells on PCAS

After 1 day of incubation, PC12 cells were attached to the area with polylysine coating in low serum RPMI medium 1640. Under this condition, cell proliferation slowed down and axon began elongation. The results of PC12 cultured on 250  $\mu\text{m}$  diameter PCAS with 400  $\mu\text{m}$  center to center spacing were shown in Fig. 7. After another day of incubation, the attached PC12 cells began to differentiate into axons. The axons were mainly grown in the area of polylysine coating (Fig. 7(a)). On day 3, the elongating axon had crossed over the gaps between the cell clusters (Fig. 7(b)). The axon successfully protruded from area with polylysine coating to non-coating area and connected to adjacent cell cluster. On day 5, the number of axons that



**Fig. 5** Optical images of patterned PDMS films and the fabricated cell arrays. The arrays are with a diameter of 150  $\mu\text{m}$  in the top row and 350  $\mu\text{m}$  in the bottom row respectively. The center to center spacing for adjacent cell colonies is 500  $\mu\text{m}$ . The structures of cell arrays fabricated by process A (Fig. 4(a)) are shown in (a) and (e). The magnified images are shown in (b) and (f). The structures of cell

arrays fabricated by process B (Fig. 4(b)) are shown in (c) and (g). The magnified images are shown in (d) and (h). Inserted images in (d) and (h) show the morphologies of cell arrays after the removal of PDMS films. White-dashed circles in inserted images of Fig. 5(d) and (h) indicate the area of polylysine coated region. Scale bars in these images are 100  $\mu\text{m}$

were connected to nearby cell clusters increased and the axons were branched out all directions (Fig. 7(c)).

### 3.5 The application of PCAS in a cell migration study

The 6 $\times$ 6 PDMS array with 250  $\mu\text{m}$  diameter and center to center spacing of 600  $\mu\text{m}$  was used in the cell migration study. After 1 day of incubation, the PDMS films were removed and the medium was replaced with DMEM containing either 0.1 % DMSO or 20  $\mu\text{M}$  Y27632. The cell morphologies of control and Y27632-treated were shown in Fig. 8(a)–(d) and (e)–(h) respectively. The initial morphologies of the PCAS after the removal of PDMS films were shown in Fig. 8(a) and (e). The morphologies of PCAS after another 12 h of cultivation were shown in Fig. 8(b) and (f). Figure 8(c) and (g) were the immunofluorescent staining of Fig. 8(b) and (f).

## 4 Discussion

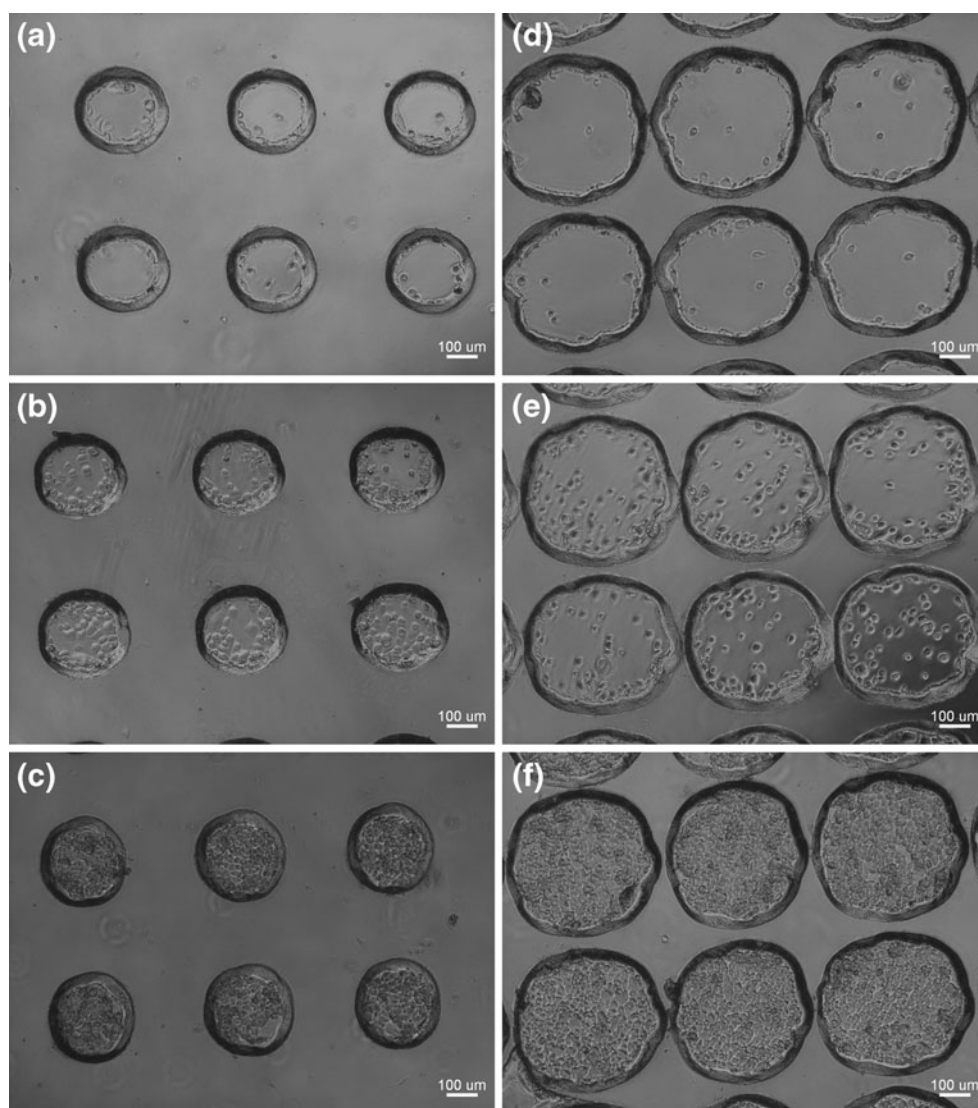
Predetermined cell patterns on a specific area with defined shapes are helpful for investigating the function of cell interaction and the migration of cell in substrate. Extracellular matrix protein microarray can be used to identify matrix compositions that would mediate attachment of specific cell types (Woodrow et al. 2009). Microwell array system is a potentially versatile tool for biological research such as embryoid body formation and evaluation of quiescent hematopoietic progenitor cells for high-throughput stem cell

experimentation (Fujita et al. 2008; Moeller et al. 2008). The use of layer-by-layer deposition of biopolymers provides an established co-culture system for studying cell behaviors such as cell-cell communication and cell-matrix interactions (Fukuda et al. 2006). Furthermore, cell migration genes can be screened on-chip using a transfection microarray (Onuki-Nagasaki et al. 2008). Cell patterning technology had opened up a wide range of application in cellular research. Thus developing a simple, cost effective, and user-friendly cell patterning method is important for future studies.

Laser sintering of hydrophobic PDMS films provides a new fabrication method for cell patterning. The pattern can be designed on computer skew software and output to the laser micromachining device. Robotic laser arrayer then produces reproducible pattern on PDMS films. The patterned PDMS films can then be attached to TCPS or glass and used to form cell arrays after cell seeding and culturing. The finest resolution in the CO<sub>2</sub> laser sintering is about 50  $\mu\text{m}$ . In cell biology, the cell arrays are valuable for evaluating cell function in this length scale. Cell arrays of higher precision can be obtained using high resolution laser. In this study, we focus on simplifying the fabrication process of micrometer scaled cell arrays.

The adherent fibroblast cell arrays were produced without any modification on the culture substrate. Cell array colonies of 100  $\mu\text{m}$  and 350  $\mu\text{m}$  diameter were fabricated after 3 h of cultivation. Cell clusters grew and maintained the morphology in the patterned microwells of PCAS (Fig. 3). After 2 days of incubation, cells on the pattern grew to confluent and formed compact cell clusters.

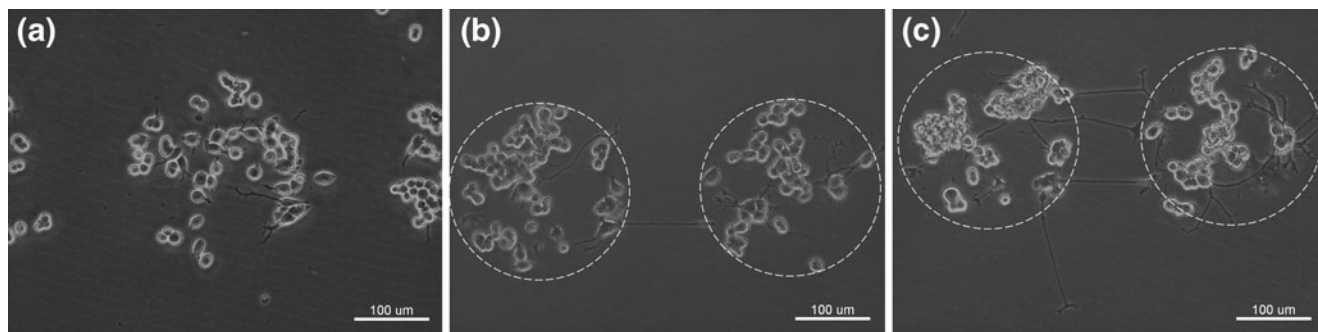
**Fig. 6** Optical images of the fabricated cell arrays with different cell seeding density. The arrays are with a diameter of 150  $\mu\text{m}$  in left column and 350  $\mu\text{m}$  in the right column respectively. The center to center spacing is 500  $\mu\text{m}$ . The morphologies of cell arrays with  $5 \times 10^1$ ,  $5 \times 10^2$ , and  $5 \times 10^3$  cells/ $\text{mm}^2$  are subsequently shown in (a), (d) and (b), (e) and (c), (f). The unattached cells were removed after 3 h of incubation. All scale bars in these images are 100  $\mu\text{m}$



After removing the PDMS films, the morphology of cell cluster remained unchanged.

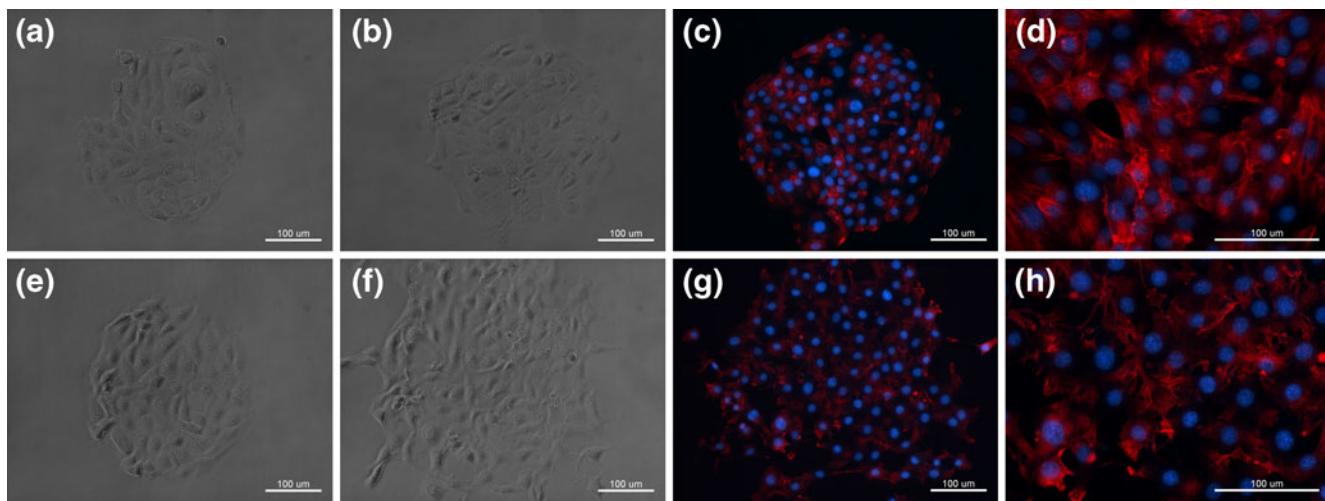
To accelerate the formation of cell arrays, we investigated the effect of initial cell seeding density on the behavior

of cell array colonies. At low cell density ( $5 \times 10^1$  and  $5 \times 10^2$  cells/ $\text{mm}^2$ ), the number of cells in the patterned wells was measured to be higher than the theoretical cell density (total cell number / surface area of culture well). At high cell



**Fig. 7** The morphologies of differentiated PC12 cell array. The array has a diameter of 250  $\mu\text{m}$  and center to center spacing of 400  $\mu\text{m}$ . Cells began to grow axon in the area of polylysine coated region after 1 day of cultivation with low serum RPMI medium 1640 (a). The

axon differentiated from one cell clusters to another and connected the two clusters after 3 day of differentiation (b). Axons branched out in different directions are observed after 5 days of differentiation (c). All scale bars in these images are 100  $\mu\text{m}$



**Fig. 8** Optical and fluorescent images of cell arrays in Y27632 cell migration study. The  $6 \times 6$  PDMS array with a diameter of  $250 \mu\text{m}$  and center to center spacing of  $600 \mu\text{m}$  was used in cell migration test. (a)–(d) are the results of the control groups and (e)–(h) represented the results of Y27632-treated groups. The initial morphologies of cell

arrays are shown in (a) and (e). The morphologies of cell arrays after 12 h of cultivation are shown in (b) and (f). (c) and (g) represent the immunofluorescent staining for (b) and (f). Magnified images of (b) and (f) are shown as (d) and (h). All scale bars in these images are  $100 \mu\text{m}$

density ( $5 \times 10^3 \text{ cells/mm}^2$ ), the number of cells in the patterned wells was measured to be lower than the theoretical cell density after 3 h of incubation. Cells attached to the culture plate and occupied the area inside the patterned microwells to form crowded cell clusters. The distribution of cells inside the patterned microwells revealed that for fabricating compact cell array, the cell density ranged from  $5 \times 10^2$  to  $2 \times 10^3 \text{ cells/mm}^2$ .

For poorly adherent PC12 cells, a modified protocol should be used as compared to adherent cell. Two different processes could be used for cell array fabrication, as shown in Fig. 4. The two methods revealed different cell array morphologies. The cell density after cultivation was higher in process A where PDMS films were removed prior to seeding compared with process B where PDMS films were not removed (Fig. 5). Different cell densities can be adapted to different applications. For example, cell-cell interaction requires compact cell morphology with suitable cell contacts. However, for differentiation of PC12 cells, free individual cells on the patterned arrays are desirable, where as highly compacted cell distribution will deteriorate the differentiation of axons in neuron cells. The cell patterns formed with PDMS films being removed prior to seeding had shown a more confluent occupation on the patterned areas (Fig. 5 (a), (b), (e), and (f)). If PDMS films were not removed, cells were randomly anchored to the culture well (Fig. 5 (c), (d), (g), and (h)). Although the boundary of cell arrays was not clear after the removal of PDMS films (as shown in inserted figure on Fig. 5(d) and (h)), in this differentiation experiment, patterned PC12 cells still successfully

differentiated into axons. The length of these axons could reach more than  $300 \mu\text{m}$ , which was long enough to connect nearby cell clusters (Fig. 7).

In this work, we demonstrated a simple, low cost, and fast method to pattern cells on the selected substrate. This platform could be used in fabricating cell arrays for both adherent and poorly adherent cells. Controlling the initial seeding density provided a manner to control the numbers of cell inside each patterned microwells. Fine cell arrays can be produced in a very short time which is equivalent to the time needed for cell anchoring (Fig. 6). In the PC12 differentiation experiment, we observed that the differentiated axon initially grew on the polylysine coated region (Fig. 7a). However, the elongated axon crossed over the non-coated region and bridged nearby cell clusters together (Fig. 7b). After continuing cultivation, different directions of axon differentiation were observed. Comparing the axon morphology in the PCAS with the non patterned polylysine coated culture plate (figure not shown), there were fewer branches of axons in the PCAS. The factors that mediated directional axon elongation were not discussed in this paper. Y27632, an inhibitor of ROCK (Rho-associated kinase), was used in the cell migration test. Previous study indicated that in the presence of ROCK inhibitor, cell migration was accelerated but polarization of both the Golgi and MTOC were inhibited in NIH/3T3 cells (Magdalena et al. 2003). Using PCAS, growth and detailed morphologies were imaged through fluorescent staining (Fig. 8). We can easily differentiate the morphological difference of treated cell colonies (8F, 8G, 8H) and untreated cell colonies (8B, 8C,



8D). Cell under Y27632 treatment revealed long microtubules and spread faster, as the same results shown in the paper of Magdalena et al (Magdalena et al. 2003). This cell array platform provided a simple method to observe this phenomenon with a large number of duplication using simple 24-well culture plates.

PCAS is useful for studying the cell functions between localized cell clusters in two dimensions. Patterned cell arrays are potentially useful as a model for studying the behavior and development of highly ordered neural networks, and can be used as a tool for high throughput drug screening or cell-microenvironment interactions. Co-culture system can be easily accomplished by sequentially seeding of different types of cells.

## 5 Conclusion

In contrast to photolithographic techniques, automatic laser micromachining on hydrophobic PDMS films provides simple, fast and cost effective fabrication of patterned PDMS arrays. We had successfully produced cell arrays for adherent cells, such as NIH/3T3, and for poorly adherent cells, such as PC12 cells, on TCPS. In the differentiation experiments using PC12 cells, we observed axon elongation of the inducing PC12 cells on cell array and the successful connection between two cell clusters through these elongated axons. The cell migration tests also showed significant changes in cell array morphology after treatment with Y27632. The cell array fabricated by this method is easily achieved and can be adopted to study cell-cell interaction, cell-substrate interaction or for drug screening.

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