Novel three-dimensional porous film for single-cell microarray applications

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ABSTRACT

Cell-based studies yield data averaged over a large number of cells which overlook the rich information that can possibly be obtained from single-cells study. For this reason, it is imperative to develop single-cell microarray to study each cell individually. However, the current techniques for generating single-cell microarray by using electromagnetic forces needs special medium and may have unknown effects, whereas mechanical methods are unable to trap cells with different sizes and are slow. Efficiency is also a concern for current techniques. Here, we demonstrate an anti-clogging 3D porous film integrated with pyramidal microstructures for generating single-cell microarray. An interesting feature of this 3D film is that cells of different sizes can be trapped. High density single-cell microarray with efficiency and cell-viability close to 90% has been achieved in this device.

Keywords: single-cell, microarray, porous film

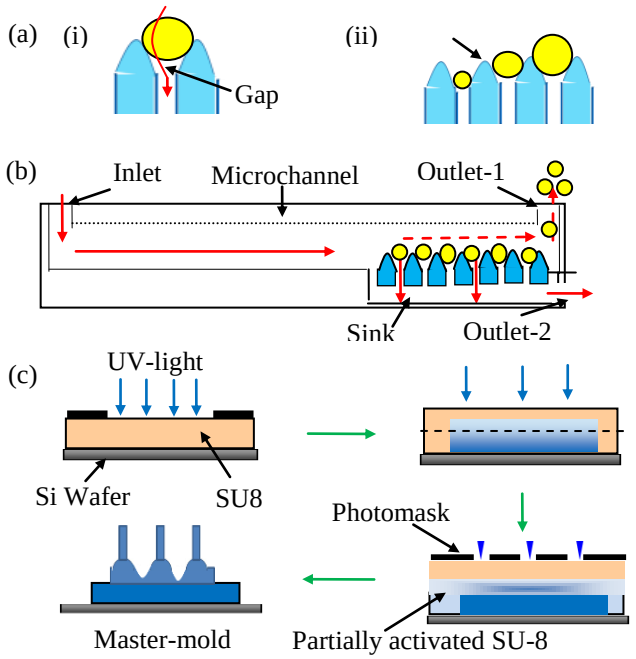
1 INTRODUCTION

A cell is a dynamic system which involves a number of biochemical reactions for its survival and for response. The stochasticity of biochemical reactions determines cellular response at an instant and thus makes cell unique in their response. Cell-based studies yield data averaged over a large number of cells which overlook the rich information that can possibly be obtained when cells are studied individually. Also, parameters that are measured as averages of large populations can be misleading due to inherent cellular heterogeneity [1]. For this reason, much emphasis has been placed over the past decades to develop single-cell microarray to study each cell individually [2]. Thus, there is a great need to develop technologies to study a number of single-cells individually.

Different techniques have been used for trapping single-cells by using either electromagnetic forces (like electrical, magnetic and optical) or by using mechanical methods (like physical or chemical traps) [3-6]. However, the current techniques for generating single-cell microarray by using electromagnetic forces needs special medium and may have unknown effects. For example, dielectrophoresis can be used for trapping single-cells individually [7], but the use of dielectrophoresis is limited by its stringent requirement of special type of medium solution with specific electrical properties, cost, use of electrical field which may affect the physiology of living cells and the trapping is sensitive to the flow speed which limits its throughput. Mechanical methods of trapping single-cells are simpler. Mechanical traps using pillars, wire, 'C' shaped structures can be used for this purpose, but they suffer from low throughput, low efficiency and unclean separation [5]. Recently, 'C' or 'U' shaped microstructures have gained popularity as mechanical trap to obtain an array of single cells [8]. However, such structure tends to trap cells of certain size and hence compromises on the heterogeneity of cell trapped. Moreover, such design limits the density at which an array can be designed and are unable to create a cell array with high density. In another strategy, cells are loaded in cell-sized depressions or wells [6]. Though, such strategy may create a cell pattern of high density single-cell array, but docking of cells in micro wells is not an efficient process and the microenvironment around a cell may get altered due to lack of reagent exchange.

Here, we demonstrate an anti-clogging 3D porous film which consists of an array of funnel-like pores where each pore is surrounded by four 3D pyramidal microstructures. Such an arrangement creates unique trapping sites in the film which helps in trapping single-cells individually without blocking pores. The trapped cells are being held by these microstructures leaving interstitial-gaps between trapped cells and pores and do not block pores (Scheme1ai). Pressure-drop can be negated due to non-blocking of pores. The 3D pyramidal microstructures help in trapping cells of different sizes (Scheme1aii) which is important for trapping single-cells as cells have inhomogeneous size-distribution. Each trap can only capture a single cell and the untrapped cells can be washed-off from the device (Scheme1b). High density single-cell microarray can be achieved in this device. The cell viability in the device has also been studied here.

A new method has been developed here to fabricate 3D micro-structures (Scheme1c). The method is based on partial activation of a photoresist (SU-8) by low-dose of exposure energy and molecular self-assembly of partially activated molecules to produce complex 3D micro-structures. The 3D microstructures fabricated by this method are replicated in thin porous film. A new solvent based approach has been developed in this project for such replication. This approach allows easy release of the porous film from the master-mold without any damage.



Scheme1: The idea- (a) (i) the scheme represents the idea of using three-dimensional pyramidal microstructures surrounding each funnel-like pore in the porous film for single-cell capture. (i) A trap showing trapped cells which do not obstruct the fluid flow. (ii) Cells of different sizes can be singly trapped. (b) Each trap is occupied by a single cell only; the untrapped cells are washed-off through the outlet-1. (c) The technique for developing such a film is represented here.

2 MATERIALS AND METHODS

Reagents and materials: SU-8 2050 photoresist and developer (MicroChem), PDMS (Sylgard-184, Dow Corning) were purchased. (Tridecafluoro-1,1,2,2-tetrahydrooctyl)-1-trichlorosilane, Isopropyl Alcohol (IPA), Calcein-AM and Propidium Iodide (PI) were purchased from Sigma–Aldrich (Singapore). Cancer cells (MDA-MB-231) were purchased from ATCC (Manassas, VA). Basal DMEM cell-culture media and fetal-bovine serum (FBS) were purchased from Invitrogen (Singapore).

Master-mold fabrication: A SU-8 layer was spin-coated at 4500rpm on a silicon-wafer, soft-baked and was exposed to UV-light through a photo-mask (designed for the channels) by a mask-aligner (Karl-SUSS Micro Tec.). This layer served as replica for channel fabrication. Second layer is then coated and is exposed by UV-light with low-dose of exposure-energy (7mJ/cm^2) and the third layer is coated which is exposed again through the photo-mask (array of circles for making micro-pillars). After final PEB, it is developed to obtain a master-mold.

Device fabricatio and Sink-layer fabrication: The PDMS device as well as sink-layer was fabricated as described in other paper in the same conference proceeding with title "*Capture of circulating tumor cells (CTCs) using a novel micro-device"* to be presented at 11:20pm,19th June-2012.

Anti-clogging study: To study anti-clogging of the 3D porous film after trapping cells, different sizes of beads were flowed after cell patterning and their flow was compared.

Single-cell microarray: Cancer cell-line MDA-MB-231 was cultured in a DMEM cell culture-medium supplemented by 10% FBS in culture flasks. Cells were trypsinized and were counted using a hemocytometer. Cancer cell-line MDA-MB-231 was cultured in a DMEM cell culture-medium supplemented by 10% FBS in a cell-culture flask. Cells were trypsinized and were counted using a hemocytometer. Cell solution was diluted in complete culture medium to the concentration of 10^5cells/mL. 100µL of the cell mixture was flowed in the device at a flow rate 50µLmin^{-1}. Unpatterned cells were removed from the device by washing.

Cell Viability: The device was sterilized by UV-light for 15min and by flowing 70% ethanol through channels. It was rinsed by 1X PBS for 15min. Then cell-culture media was flowed in the device and it was allowed to stand for 30-60min before starting cell-based experiments. A solution containing 10µM Calcein-AM and 10µM PI was prepared in 1X PBS for labeling viable and non-viable cells. Cells were patterned as before. The cell-staining solution was flowed in the device and it was incubated for 15min at ambient temperature and fluorescence images were captured by using a fluorescence-microscope (Nikon Eclipse-80i). Cell viability was assessed by counting viable/non-viable cells on the device using "ImageJ" software.

3 RESULTS AND DISCUSSION

3.1 Device Fabrication

The master-mold for the fabrication of the desired 3D porous film has been achieved by the method described here (Fig.1a). Interestingly, this method has allowed fabrication of 3D microstructures by photolithographic approach. This master-mold is used for the fabrication of the microfilter device which consists of a short microchannel connected to the porous film. The sample can be flowed in the film through this microchannel. The flow-through flows out in a sink just below the film (Scheme-1b). This device has been successfully fabricated by the method mentioned here (Fig.1). The film can sag during fluid flow which may not be suitable for imaging. Thus, 'Doll-shaped' flexible microstructures have been fabricated in the sink to support the film (Fig.1e). To peel-off the film from the master-mold, a solvent-based approach as described in the previous section has been applied for easy release of film from the mold. The film consists of a well-arranged array of 6µm pores separated by 15µm from each other. Each pore is surrounded by four pyramid-shaped microstructures (base-diameter 10µm and height 10µm).

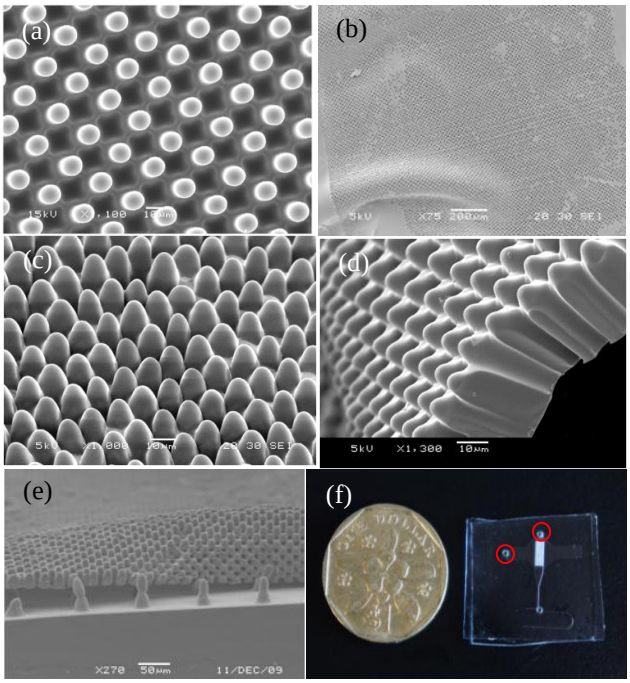


Figure1: The Porous Film: SEM images showing (a) the SU-8 master-mold fabricated by the developed method, (b, c, d) are bottom, tilted and side view of the porous film respectively. (e) PDMS film resting on the unique 3D 'doll-like' microstructures that are fabricated in the sink to support the film. (f) The final PDMS device kept near one Singapore-dollar coin for size comparison. The circles show oultes in the device.

3.2 Anti-clogging Study

As shown in the Scheme-1a, trapped cells should not block pores due to interstitial gaps between trapped cells and pores. To prove this hypothesis, beads of different sizes (all smaller than pore-size) were flowed under similar conditions through the film with patterned cells. The percentage of beads passing through the film was plotted (Fig.2). Reasonably, beads smaller than interstitial-gaps should pass through these gaps while beads bigger than interstitial-gaps should settle around patterned cells as fluid passes through these gaps. Now, if the size of all sizes of beads are smaller than pore-size, there should be a significant difference between percent of these beads passing through the 3D film under same experimental condition. As seen from the graph, the percentage of 5µm beads passing through the film is low compared to other two sizes of beads. This can happen as 5µm beads are unable to pass through the interstitial gaps while smaller beads should have passed through. The 5µm have been found settled around patterned cells while smaller beads have been found in the flow-through. Moreover, no beads have been seen in areas without any cells. This indicates that the pyramidal microstructures confer anti-clogging characteristics.

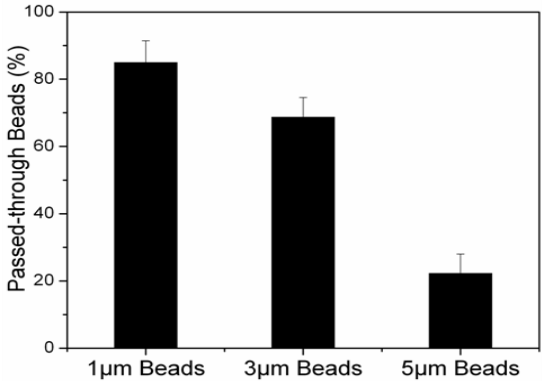


Figure2: Anti-clogging: The plot showing percent of beads of different sizes passing through the microfilter with patterned cells.

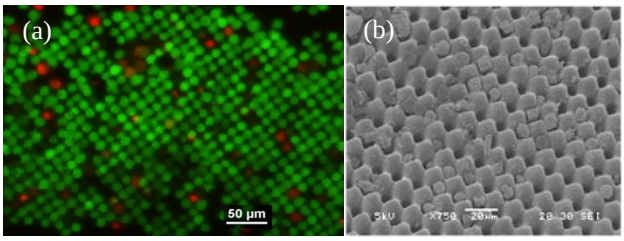


Figure3: Single-cell Microarray: (a) Fluorescent image showing high density of single-cell microarray. Green and red color indicates live and dead cells respectively, (b) SEM image showing singly patterned cells on the film.

3.3 Single-cell Microarray

The 3D pyramidal microstructures offer two clear advantages over any other device for cell trapping. First, cells with different sizes can be trapped here due to slop of pyramidal microstructures. Second, untrapped cells can be washed-off easily which help in obtaing individually patterned cells. As shown in Scheme-1b, the untrapped cells can be washed away through outlet-1 as trapped cells retain their position during washing. Due to these characteristics of the 3D porous film high efficiency of single-cell patterning (~85%) has been achieved in this work where patterning efficiency is calculated by the given formula:

$$Patterning\ \ efficiency \equiv \frac{Total\ No.of\ individually\ trapped\ cells \times 100}{Total\ No.of\ cells\ used}\ \%$$

Figure-3 shows the single-cell microarray obtained on the 3D porous film. To obtain such microarray, cells are flowed at flow-rate of 50µl/min in the device. Untrapped cells are washed away and the process is repeated to trap maximum number of cells. The whole process is completed in less than 15min. Fluorescence as well as SEM images of patterned are obtained. The trapped cells in this SEM image looks smaller than their normal size (14-20µm) which is probably due to shrinkage of cells during sample preparation for SEM (fixation and dehydration).

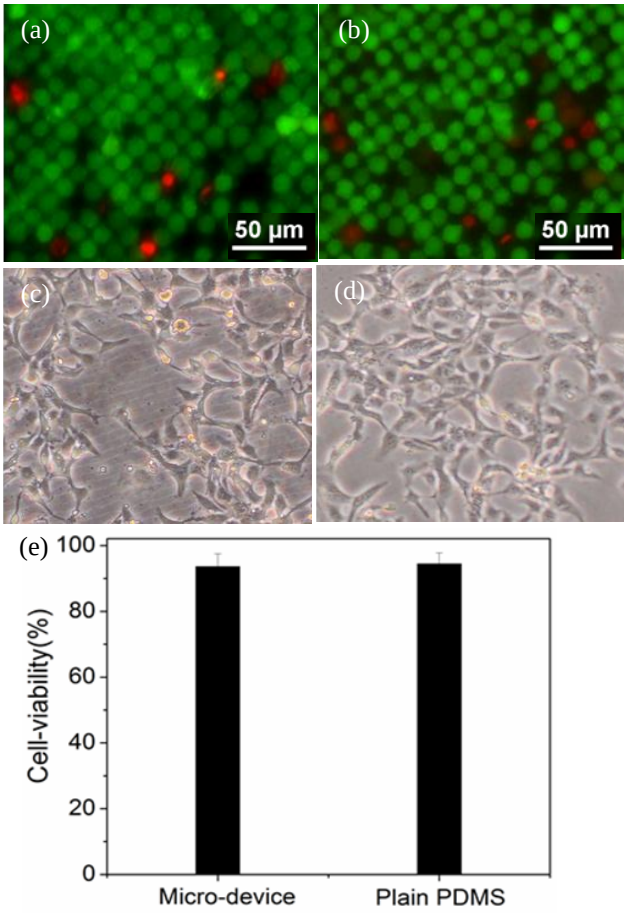


Figure4: Cell viability in the device: (a) and (b) are obtained from the cell-viability study on the device. Green fluorescence indicates viable cells and red fluorescence indicates non-viable cells. (a) Cells just after patterning and (b) after 5hr of incubation in the device. (c) Cells retrieved from the 3D porous film were cultured and (d) normal cell culture. (e) the plot showing cell viability over 5hrs of incubation in the micro-device and in a plain PDMS chamber

3.4 Cell Viability

A combination of two fluorescent-dyes Calcein-AM and propidium iodide (PI), was used here for cell viability study. Calcein-AM labels live-cells, whereas PI is used for labeling dead-cells. To perform this experiment, cells are patterned on the device and cell-viability is assessed. It has been found that cell viability did not reduce significantly even after five hours of incubation (Fig.4a & b) which proves that patterning in this microdevice does not affect cell-viability significantly. To further confirm the finding and to prove that cells remain functional, cells are flushed-out from the device after patterning by using back-flow of fluid. The recovered cells are then cultured and this cell-culture is compared to the normal cell-culture. The cells have been found to grow normally and are morphologically similar to cells from normal culture (Fig.4c, d). This experiment shows that cells remain viable and functional in this micro-device.

The cell viability in the device is also compared to the cell viability on the plain PDMS to show pyramidal microstructures are not detrimental to cells. No significant difference between cell viability has been observed in the microdevice compared to the plain PDMS (Fig.4e).

4 CONCLUSIONS

The 3D porous film is found to be anti-clogging and can be used for obtaining high density of single-cell microarray at high efficiency retaining their viability.

5 POSSIBLE IMPACTS

The porous film developed in this research can be used for obtaining single-cell data from a large number of cells simultaneously which would help in basic understanding of cell behavior with possible impact in cancer biology and drug testing. The other possible impact of this technology can be in field of biosensing as cell-based biosensor.

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