MONOLITHIC 3-D MICROFLUIDIC DEVICE FOR CELL ASSAY WITH AN INTEGRATED COMBINATORIAL MIXER

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Abstract: We present a novel cell culture device with an integrated 3-D microfluidic mixer for screening the combinatorial effects of multiple compounds on cultured cells and demonstrated the ability to simultaneously treat arrays of cells with different combinations of compounds. The proof–of-concept chip, which has a three-input combinatorial mixer with eight micro culture chambers, was monolithically fabricated utilizing the surface micromaching of parylene C (poly-para-xylylene C). By having several microfluidic "overpasses" to allow one microfluidic channel to cross over other microfluidic channels, the combinatorial mixer was able to simultaneously generate all the combinations of the input fluidic streams for output to the microchambers. We verified that parylene C is a biocompatible substrate for cell culturing and also showed cell culturing inside the micro culture chambers. The feasibility of using the integrated combinatorial mixer for cell assaying was demonstrated with experiments using three different cell stains, and results showed the cells in different chambers were stained with varying color patterns.

Keywords: 3-D Microfluidic, Parylene, Cell Culture, Combinatorial Mixer

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1. INTRODUCTION

Cells are sustained in complex environments and cell fates are dictated by the integration of numerous extracellular signals. Therefore, drug screening and biological assays often include multiple combinations of different compounds. However, as the number of compounds to be tested expands, traditional screening tools such as robotics and multi-well plates become expensive and complicated to implement. Microfluidic devices provide potentially inexpensive tools for high-throughput cell-based assays. Many lab-on-chip devices with cell culturing functionalities have been developed, but most devices can only expose cells to a single compound at once [1, 2]. The complexity of on-chip cell-based assay experiments can be greatly expanded by developing a cell-culture chip with an integrated combinatorial mixer to screen for the combinatorial effects of compounds on cells.

In this work, we developed an integrated method to monolithically fabricate 3-D microfluidic networks and fabricated an on-chip cell culture device with an integrated combinatorial mixer. Our fabrication method is simple and favorably avoids multilayer bonding process that is used in most other 3-D microfluidic fabrications [3, 4]. The device has a three-input combinatorial mixer, which simultaneously delivers 8 combinatorial output streams to the culture chambers. We cultured cells with this device and also demonstrated a simple cell assay with the integrated combinatorial mixer.

2. DESIGN

Fig. 1 shows the design layout of the 1 cm by 1 cm chip. The cell culture array consists of eight individually isolated micro-culture chambers with cell loading inlets that enable cell introduction into the culture chamber. The chamber dimensions were L 1500 µm x W 700 µm x H 32

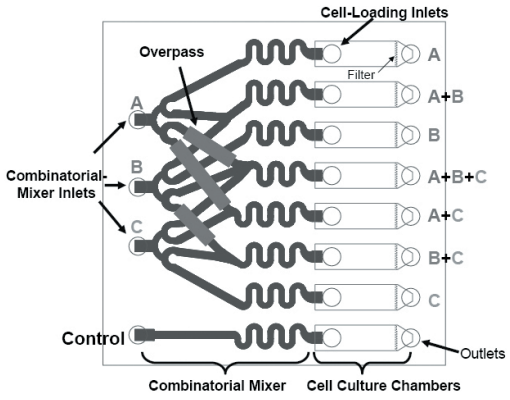

Fig. 1 Design layout of device

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µm, which corresponds to a volume of 33 nL. The device has a combinatorial mixer with three inputs, and those inputs are recombined into the seven possible outputs for delivery into the chambers. For example, compounds A, B, and C, will be recombined into A, B, C, A+B, A+C, B+C, and A+B+C. One control channel that receives an unmixed input stream is included. The combinatorial mixer has several "overpasses" that allows one microfluidic channel to cross over other microfluidic channels.

3. EXPERIMENTAL

3.1 Biocompatibility test for parylene C

To verify that parylene C is a compatible substrate for cell culturing, B35 rat neuroblastoma cells were grown on three substrates: 1) unmodified standard polystyrene Petri dish, 2) Petri dish coated with parylene C (5 µm), 3) Petri-dish coated with parylene C and pre-treated with 0.05 % Polyethyleneimine (PEI) solution. PEI solution has been shown to enhance cell adhesion to substrates [5]. The cell growth rate was modeled using the exponential model: $N(t) = N_o * \exp(\mu t)$, where N is the cell concentration, N_o is the initial cell concentration, t is the time and µ (1/hour) is the specific growth rate. By culturing the cells through certain duration, t_f and measuring the starting and final cell concentration, N_o and N_f, the specific growth rate, µ (1/hour), can be derived as $\mu = \ln(N_f / N_o) / t_f$.

3.2 Device fabrication

The device has two microfluidic levels, and the fabrication process is shown in Fig. 2. A first thin adhesion layer of parylene C was deposited (3 µm). The first sacrificial photoresist layer was spin-coated (15 µm) and patterned to define the first-level channels. A second layer of parylene C (10 µm) was then deposited to cover the sacrificial photoresist, forming the first-level channels. Parylene C was patterned using oxygen plasma so the areas where the overpass structures could be joined were exposed. This parylene patterning also opened the area where the mixer and the culture chamber would be connected. A second sacrificial photoresist was spin-coated (32 µm) and patterned to define the overpass structure and the culture chamber. This second sacrificial photoresist covered the areas that were etched open, and the overpasses spanned several of the first-level microfluidic channels. A third layer of parylene C (10 µm) was deposited and patterned to define the access holes. The whole chip was planarized with thick SU8 (100µm). Finally, the chips were soaked in IPA to dissolve the sacrificial photoresist.

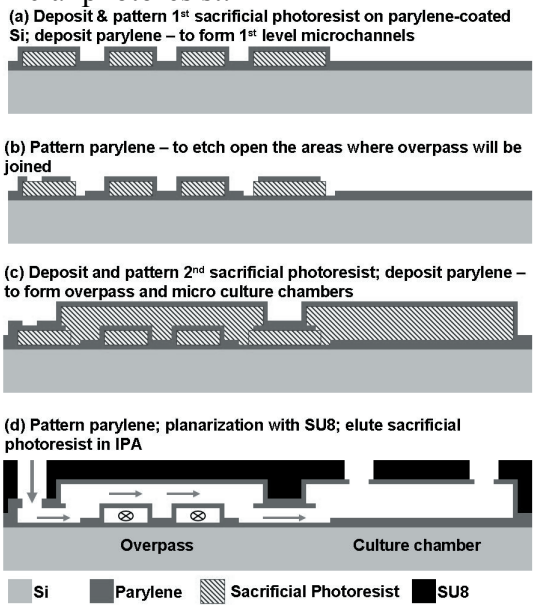

Fig. 2.Microfabrication process. Arrows in (d) shows that two fluid streams are separated spatially at the overpass region and flow in two different directions (→ and ⊗).

3.3 Packaging and device operations

A packaging scheme was developed to make the fluidic connections with the chip as shown in Fig. 3. A piece of cured PDMS with punched holes was aligned onto the chip. The PDMS and the chip were clamped together by two pieces of transparent acrylic that were milled. The PDMS acted both as a gasket layer to provide proper sealing and also as a port to receive the tubes. Depending on the operation of the device, the PDMS piece can have holes open at different places to either close or open certain access holes on the chip. To test the combinatorial mixer, the device was packaged with holes open at the combinatorial-mixer inlets and outlets, with the cell-loading inlets closed, as shown in Fig. 3(b). Green, red, and blue food coloring solutions were injected into the three inputs of the combinatorial mixer, while yellow food coloring was delivered into the control channel.

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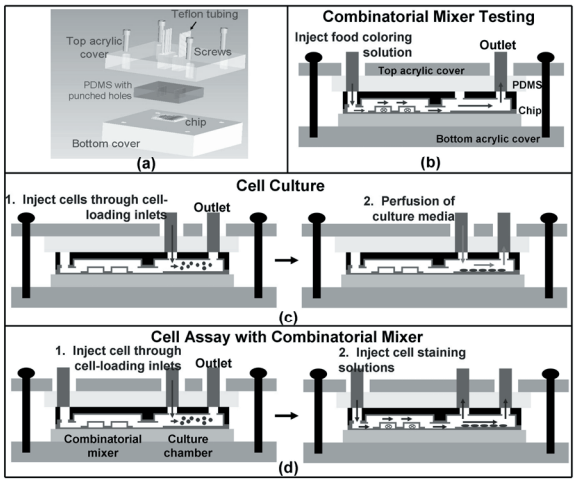

Fig. 3 (a) Schematic representation of the packaging method. Chip packaging for various device operations: (b) combinatorial mixer testing, (c) cell culturing experiment, (d) cell assay with combinatorial mixer.

To culture cells on chip, the device was packaged with the cell-loading inlets and outlets open (Fig 3(c)), and the culture chambers were treated with 0.05 % PEI solution to enhance cell adhesion. Cells were delivered into the culture chambers and allowed to attach to the culture chamber surface for 4 hours. Cells were cultured with continuous perfusion of culture media at flow rate of 33 nL/min.

A simple cell assay was performed using the integrated combinatorial mixer. The device was assembled with all the inlets and outlets open (Fig. 3(d)). After cell loading and attachment, three different cell staining solutions (0.005% crystal violet, 0.08% neutral red and 0.01% methylene blue) were injected through the combinatorial-mixer inlets. No cell stain was injected into the control channel. The stain solution injection was controlled by syringe pumps and was set at 30 µL/min for a duration of 10 minutes. Pictures of each culture chamber were taken 30 minutes after the flow was stopped.

4. RESULTS AND DISCUSSION

The growth rates of B35 cells on different substrates are plotted in Fig. 4. The result shows that the growth rate on parylene C, 0.041/hr, is slightly slower than the growth rate on polystyrene Petri dishes, 0.049/hr. However, after the parylene C was treated with PEI solution, the growth rates on both surfaces were exhibited similar values. These results show that parylene C is a biocompatible substrate for cell culturing and is a suitable material for constructing micro culture chambers.

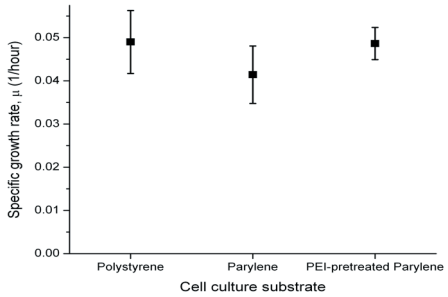

Fig. 4 B35 cell growth rates on three different substrates

SEM image (Fig. 5(a)) of the overpass structures shows the two-level microfluidic channels and such a structure could allow two fluidic streams to be separated spatially at the overpass region. Fig. 5(b) shows the combinatorial mixer as it took in the three input streams of food colorings and generated the seven possible combinations. The control channel

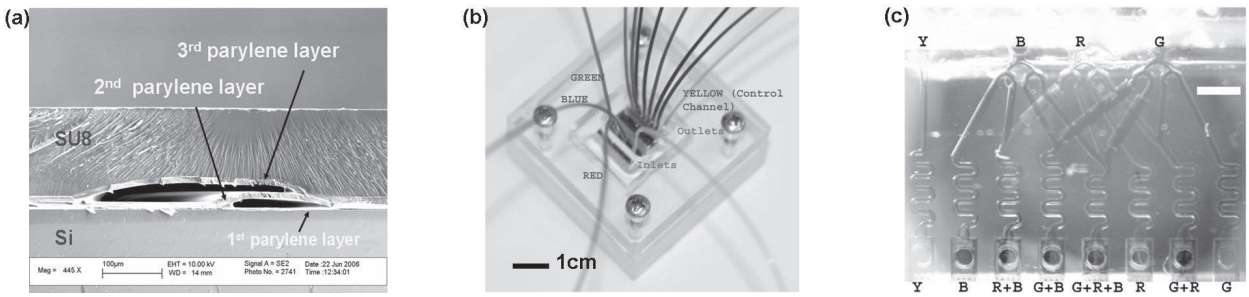

Fig. 5 (a) SEM of the microfluidic overpass. Combinatorial mixer demonstration: (b) Assembled device with food coloring being injected. Green (G), red (R) and blue (B) food colorings are injected into the combinatorial-mixer inlets, while the yellow (Y) food coloring is injected into the control channel. (c) Combinatorial mixer operated at 10 µL/min; scale bar represents 1 mm.

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received only the yellow food coloring that was not combined with any other solutions. The overpass structures allow one fluidic stream to flow over several other microchannels (Fig. 5(c)).

Cell culture inside the micro culture chamber was performed. Fig. 6 shows that cells proliferated inside the micro culture chambers and

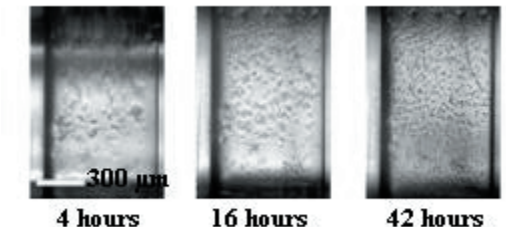

Fig. 6 Cell culture on chip. B35 cells attached to the surface 4 hours after cell loading and proliferated. Pictures were taken 4 hours, 16 hours and 42 hours after cell loading.

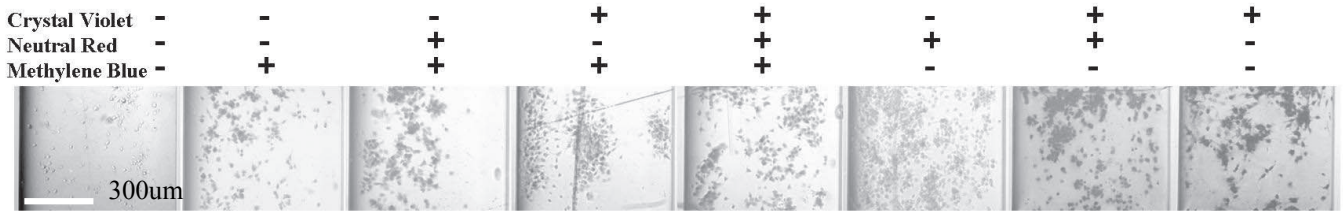

Fig. 7 Cell assay with combinatorial mixer. Three stains were injected, and cells in different chambers received different combinations of dyes and were stained with different color patterns.

eventually reached confluency.

A simple cell assay using the integrated combinatorial mixer was demonstrated and the result is shown in Fig. 7. Following cell injection and adhesion, three cell stain solutions were injected through the combinatorial-mixer inlets, while no solution was injected into the control. The combinatorial mixer simultaneously delivered the various combinatorial streams into the cell culture chambers. Cells in different culture chambers received different combinations of the stains and were stained with different color patterns, while the cells in the control chamber were unstained. This simple experiment shows that we can use the on-chip combinatorial mixer to simultaneously interrogate individual culture chambers, and each group of cells inside the different culture chamber received different inputs and showed distinct responses.

5. CONCLUSION

In this work, we demonstrated the monolithic fabrication of an integrated combinatorial mixer for cell assay, and showed the ability to simultaneously treat arrays of cells with different combinations of compounds. Based on this technology, chip with high-density cell array and integrated high-input combinatorial mixer can be constructed for high throughput cell assay, which can efficiently collect large quantities of information pertaining to cyto-regulation and elucidate combinatorial effects of multiple compounds on cells. Such device could significantly impact a spectrum of fields including the pharmaceutical industry, systems biology and stem cell studies.

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