MICROPATTERNING OF HYDROGEL AND ON-CHIP LONG TIME MONITORING OF INDIVIDUAL CELLS IN A CAGE

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ABSTRACT

We developed a novel technique to observe individual cells in various environments using a cage made of patterned hydrogel on polydimethylsiloxane (PDMS) microfluidic chips. The hydrogel is polymerized by UV-ray illumination (300-400 nm) on a glass surface, which is spin coated with the water-soluble prepolymer solution, and immobilized on the glass surface. The hydrogel is hydrophilic, transparent, biocompatible. Then, the PDMS microchannel is assembled and bonded on it. We transported cells into a cage using optical tweezers. In the cage, solution flow is stable and chemicals permeate through the wall, so cells are not flowed and we can change chemical conditions around cells stably. We succeeded in making a system to observe cells stably for long time.

KEYWORDS: Cell Handling & Analysis, Hydrogel, Cage, Optical Tweezers

INTRODUCTION

There are great demands to investigate unknown properties of microorganisms and cells. Analysis based on the batch culture has been commonly used, however, we can get averaged information of specimens. Therefore single cell analysis on a chip has been studied actively. If we can control environment around specimens and monitor behavior one by one for long time, we can measure dynamic change of the specimens. However, it is difficult to change environmental conditions without losing sight of specimens. To solve this problem, there have been reported on a cage made of permeable membrane [1],[2], however, conventional methods are not easy to realize fine patterning of vertical permeable walls. Here we succeeded in patterning permeable walls on glass surface by photolithography. The proposed method is suitable to make any 2D structures in microchannels.

METHOD OF CAGE FORMATION

A cage is made of UV patterned hydrogel (BIOSURFINE®-AWP, Toyo Gosei Co., Ltd) in polydimethylsiloxane (PDMS) microchannel. Figure 1 shows mechanism of hydrogel formation by UV exposure. Bonding force is strong enough for microfluidic experiments (flow speed < 2000 μm/s). This hydrogel is water –soluble and we can make any pattern on glass surface by lithography [3]. The patterned hydrogel formation has a good water-absorbing property and it is easy to dye by water-soluble chemicals.

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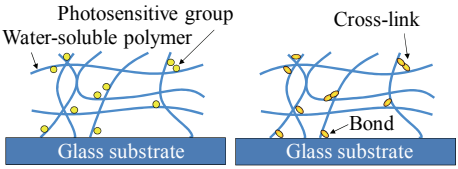
Figure 2 shows schematic of long time cell observation system with solution changing. A cell (synechocystis sp. PCC6803) was transported into a cage made by patterned hydrogel using optical tweezers [4] (a). A cell stays inside the flow stable area during and after solution changing (b). Figure 3 shows process flow to fabricate a micorofluidic chip. The channel height is about 2 μm. The patterning resolution of hydrogel is 5 μm. When we changed solution around the cage, the solution permeates into it. Figure 4 shows osmosing of fluorescent reagent. We changed solution from water (a) to Rhodamin B solution (b). Permeation time is less than 1 second. The speed depends on the flow speed of solution.



(a) Before exposure (b) After exposure

Figure 1. Mechanism of hydrogel formation by UV exposure.

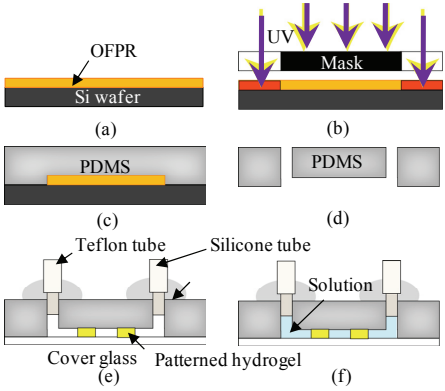
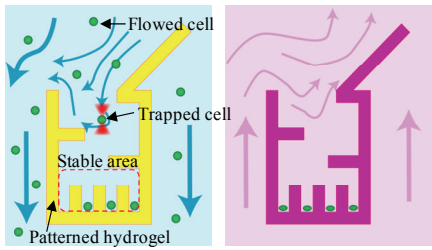
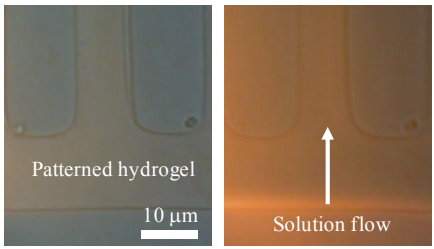


Figure 3. Process flow to produce a microfluidic chip which has cages made by patterned hydrogel. (a) and (b)Photolithography on Si wafer patterned with OFPR.(c) PDMS molding. (d) Making holes. (e) Plasma bonding with a glass surface patterned hydrogel. (f) Fixing tubes.



(a) Cell placement (b) Solution changing

Figure 2. Schematics of long time cell observation system with solution changing. (a) Cell transportation by optical tweezers into area which is walled by patterned hydrogel. (b) Solution changing.



(a) In water (b) In fluorescent solution

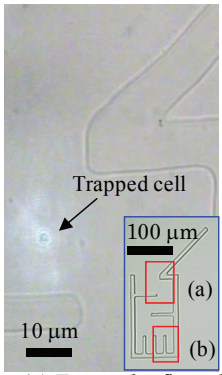
Figure 4. Fluorescent chemicals permeated into a cage. (a) No fluorescent. (b) Rhodamin B solution.

EXPERIMENTAL

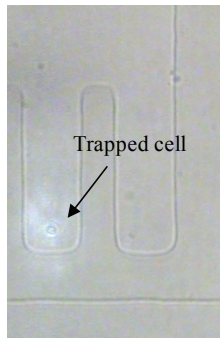
We trapped a cell near the inlet of the cage using optical tweezers and transported inside (Fig. 5). Laser power was about 2 mW and transporting time was about 20 s (a). The cell stayed inside the cage (b). We examined the laser trapped cells by using SYTOX Green after transported and they were alive(data not shown).

RESULTS AND DISCUSSION

We changed the environment around cells from water to dyed hyper osmotic solution and observe the same specimen for more than 2 hours (Fig. 6). In water, the cage was transparent and size of cells were normal (a). After solution change, the cage was stained red and cells were shrunk by hyper osmotic stress (b). Then, properties of cells are monitored in real time. The proposed system is applicable for investigation of the individual properties of cells.

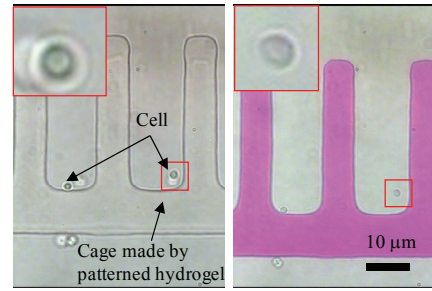


(a) Trapped a flowed cell using optical tweezers



(b) Placed the cell at flow stable area

Figure 5. A Cell transportation into a cage made by patterned hydrogel using optical tweezers.



(a) Before *(b) After solution changing*

Figure 6. A cell was shrunk by hyper osmotic stress after solution changing in a microfluidic chip. (a) In water (b) In BG11 and 3M sorbitol and rhodamine B solution

CONCLUSIONS

We succeeded in patterning permeable walls on glass surface by photolithography and made a cage for cell analysis. The hydrogel is hydrophilic, transparent, biocompatible. The proposed method is good to observe individual cells for long time.

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