UNTETHERED MICRO-PIPETTE MANEUVERED IN A CHIP BY OUTER MAGNETIC FIELD

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ABSTRACT

We have developed an on-chip micro-pipette, which has abilities of cell injection, local regent blow, enucleation, air bubble generator, piercing cells, and micro-manipulation, made by SU-8 photoresist for cloning, chimera technology, cell assays, and artificial lipid bilayer membrane making. We used the property of SU-8 which has a sufficient stiffness enough to penetrate cells as well as a sufficient softness enough to provide pumping functions, which is unlike a glass or silicon materials. Using this material, we improved our magnetically-driven micro-tool (MMT) as a multifunctional micro-tool.

KEYWORDS: Micro-manipulation, Micro-fluidics, Magnetically driven microtool, Robot on a chip

INTRODUCTION

In the field of bio-manipulation, especially for the cloning or chimera technique, it is highly required to investigate a single cell under microscope with high accuracy (µm order) to satisfy high throughput micro-manipulation. In a previous study, we developed polymer-based MMTs for cell manipulation, such as valve, droplet generation, etc [1]. These microtools were controlled by the applied magnetic fields. However, only on-off control actuation method was carried out, and the positioning accuracy was not required. In order to achieve positioning control, pairs of Helmholtz coils have been used in some research [2], however the applied force by an electromagnetic coil is not sufficiently strong to manipulate a relatively large cell such as an oocyte. On the other hand, a permanent magnet has a more than 10 times stronger magnetic field to drive an MMT than an electromagnetic coil of the same size. Our research group proposed MMTs whose minimum resolution was 1.1 µm and the response was 0.02 sec[3][4]. However, cell pipetting requires more accurately to provide cell positioning, piercing, and flow control simultaneously. For the current research, we developed a micro-tool which satisfied these abilities with SU-8 hollow structure controlled by the existing magnetically-driven system [3][4]. Figure 1 shows the overview of the on-chip micro-pipette. Left hand side micro-pipette has a large size nozzle to capture a cell. The other tool, which has a sharp nozzle, injects or enucleates. These tools are positioned by a permanent magnet, and the center of the plate are also controlled by an electrical magnet under the micro-fluidic chip.

ANALYSIS

This MMT has a novel-unique 3D structure made of multi-step exposure technique, which employ SU-8 as hollow structure, and also as a membrane pump. SU-8 membrane was suitable for pumping because the deformation is elastic. The deformation analysis of the membrane was carried FEM (COMSOL 4.0, Keisoku Engineering System Co.,Ltd) shown in Figure 2. Figure 2 (a) is the model and the schematic diagram. The radius of the pump was 2 mm, and the magnet plate was 1 mm. The thickness of the pump part was 150 µm, and the SU-8 membrane was 50 µm. The Young’s module of the SU-8 membrane was 4.3 GPa. When the loaded force was 25 mN, the maximum deformation of the SU-8 pump was about 30 µm, and hence the pumping quantity was calculated as 30.7 nl.

Figure 1. Concept of on-chip micro-pipette

Figure 2. Analysis of deformation of the SU-8 membrane

(a) Model of the analysis

(b) Result of the FEM analysis of displacement
EXPERIMENTAL

Figure 3 shows the fabrication process and microscopic images of fabricated micro-pipette. Figure 3(a) shows the design of a prototype of the micropipette, which is designed to check the pumping of the SU-8 membrane and the piercing of the cell (swing oocyte) with the SU-8 needle. The micro-pipette was composed of SU-8 membrane pump, needle, and metal plates which was actuated magnetically. The tip of the SU-8 needle was 1 μm and the nozzle had wriggle micro-channel preventing invasion of flowing water by the capillary force.

Figure 3 (b) shows the fabrication process of the micro-pipette. Alignment marks were required to be patterned (Figure 3(a)1-6) because the micro-pipette made by 3 layers exposure process.

Then, 2 layers are processed (figure 3(b) 7-12) with liquid SU-8 photo resist (Kayaku Microchem. Co., LTD., Japan). The first layer was the basement of the micropipette, and the second layer composed of the wall of the pump region and the micro-channel of the nozzle part.

One of the difficulties of the fabrication is to penetrate the path from the pump region to the tip of the needle. Eventually we had successfully established a fabrication process which produced a path (tunnel) from pump region to the tip of needle using tenting method of SU-8 sheet (50 μm). After development of SU-8 sheet, micropipette is removed from the Si wafer by the development of the LOR(lift off of photo resist).

Figure 4 shows a microscopic image of the fabricated micro-pipette by the fabrication process (Figure 3). Figure 4 (a) shows the overview and the tip of the micro-pipette. The diameter of the pump region was 2 mm, and the wriggle micro-channel was 40 μm x 50 μm. The tip nozzle of the micro-pipette was about 15 μm. To confirm the tunnel fabrication and the pumping of the SU-8 membrane, pure water was filled in the pump. Then the membrane was activated by a pusher, and we confirmed the air and the water flow from the pump region to the tip of the nozzle as shown in Figure 4 (b),(c).

1. Si wafer cleaning
2. Photoresist coating
3. Expose
4. Development
5. Cr and Au sputtering
6. Remove of photoresist
7. LOR and SU-8 coating
8. Exposure
9. Development
10. SU-8 coating
11. Exposure
12. Development
13. SU-8 seat stick
14. Exposure
15. Liftoff LOR

Figure 3. Fabrication of the micro-pipette

(a) Schematic diagram of a prototype of the micro-pipette

(b) Fabrication process

Figure 4. Microscopic images of the fabricated micro-pipette and result of pumping

(a) Microscopic photograph of the fabricated micro-pipette

(b) During operation

(c) After push
RESULTS AND DISCUSSION

Figure 5 shows the experimental results of swine oocyte piercing and pumping for confirmation of the concept of the prototype micro-pipette. Figure 4(a) is the cell piercing test using swine oocyte with zona pellucida. The stiffness of the SU-8 was enough for processing cells.

Figure 5 (b) shows an operation of pump with permanent magnet. The micro-pipette was put on the glass, and one permanent magnet was placed on the SU-8 membrane. Then the other magnet was located under the glass plate (thickness: 120 μm). The pump was loaded air, and then an air bubble was generated at the tip of the SU-8 needle part after the magnetic field applied. The pumping quantity was estimated about 5.7 nl from the volume of the air bubble. The density of the magnetic flux was about 100 mT, and the force between the magnets was about 25 mN, which was the same power calculated by the analysis (Figure 2).

These result shows that the SU-8 micro-pipette has a sufficient properties of cell piecing and pumping.

The differential of the pumping quantity between the analysis and the experiment is likely due to by the sagging of the SU-8 membrane.

CONCLUSION

We develop an on-chip micro-pipette, which has abilities of pumping and cell piercing, made by SU-8 photoresist. The pump region of SU-8 membrane was analyzed by the FEM. When the 25 mN was loaded, the SU-8 whose thickness was 50 μm was deformed about 30 μm and the pumping capacity was estimated as 30.7 nl.

We established the fabrication process using the tenting of SU-8 sheet which have a hollow structure to penetrate the path inside micro-tool. We confirmed it by the pumping test using pusher and the permanent magnet. Then the cell piercing test was also performed using swine oocyte and the result showed the SU-8 needle had enough property to process the cells.

One of the problems of this pumping method was that the permanent magnets of the pumping was attracted by the magnet for the positioning of the micro-pipette. In the future, we will solve this problem, and then we will realize the simultaneous control between the pumping and the positioning of the micro-pipette.

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REFERENCES

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