MICROMACHINED SAMPLE DIVIDER FOR ANALYZING BIOCHEMICAL REACTION BASED ON SINGLE MOLECULES

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ABSTRACT

We proposed novel type of a sample divider, which can easily divide the sample solution into multiple small amount of it on a chip, for single molecule analysis. We used the composite of a PDMS and an expancel^{R} as a material, and fabricated the sample divider structure by applying the mold process. We investigated the PDMS and the expancel mixture fraction dependency, and effect of the heating time, on the channel closing performance. The flow channels were successfully closed when the volume ratio between the expancel and PDMS solution was 1:2 and the heating time was 15 minutes. From these results, we concluded that the proposed device is useful as the sample divider, if we optimally designed.

1. INTRODUCTION

The progress of Micro-Electro-Mechanical Systems (MEMS) has opened the door for the miniaturization and the integration of various types of mechanical and electrical components onto the same chip, allowing researchers to create novel types of mechanical systems for the automotive, optical, information technologies and medical industries. Researchers are now also working to develop portable micro-chemical analysis systems (Micro-TAS), sometimes called lab-on-a-chip, for biological applications. Micro-TAS is able to reduce the amount of reagent solutions, and it also enables on-site monitoring. Many types of Micro-TAS have been proposed for biological applications [1–5].

Recently, micro-machined arrayed chambers draw the attention, as an analytical tool of the biochemical reactions based on a single molecule. Ottesen et al., developed nano-liter-volume of reaction chambers for a digital Polymerase Chain Reaction (PCR) [6]. They formed an arrayed micro-chambers, micro-channels, and micro-valve on the same chip. The sample fluid was introduced by the flow channels, and the integrated valves were pneumatically driven by the gas pressure. The system is able to provide the novel type of digital PCR, however, it requires the external bulky gas control systems for its arrayed micro-valves actuations. The structure itself is also complicated. Rondelez et al., proposed the different type of the femto-liter chambers for analyzing enzymatic reaction based on a single molecule [7]. They used the molding and the sealing process in their fabrication. They fabricated the arrayed chambers on the surface of the Poly-dimethylsiloxane (PDMS) sheet by mold, and then covered the sample droplet on the glass plate by the PDMS sheet. During the sealing process, a single molecule is stochastically enclosed into a single chamber. The structure and the fabrication process is simple, however, it is difficult for handling the sample solution at the sealing process.

We therefore proposed a new kind of a sample divider, which can easily divide the sample solution into multiple small amount of it on a chip, for single molecule analysis. The proposed divider has a great advantage that it does not need any mechanical components and complicated structure. It is also able to handle the sample solution easily.

2. OPERATION PRINCIPLE OF SAMPLE DIVIDER

A concept of the sample divider for the biochemical reaction analysis based on a single molecule is shown in Fig. 1. At first, the sample solution is extracted by a pipette, and then it is delivered onto the micromachined sample divider by droplet (Figs. 1(a)-(b)). The divider has huge numbers of chamber units, and it has a function that it can divide the droplet into the isolated multiple solutions. After dividing the solution into each chamber, the biochemical reaction is then performed on the divider (Fig. 1(c)). Then, the biochemical performance at each chamber is investigated to understand the amount of the reaction based on single molecule and individual differences. The operation principle of the divider is as follows.

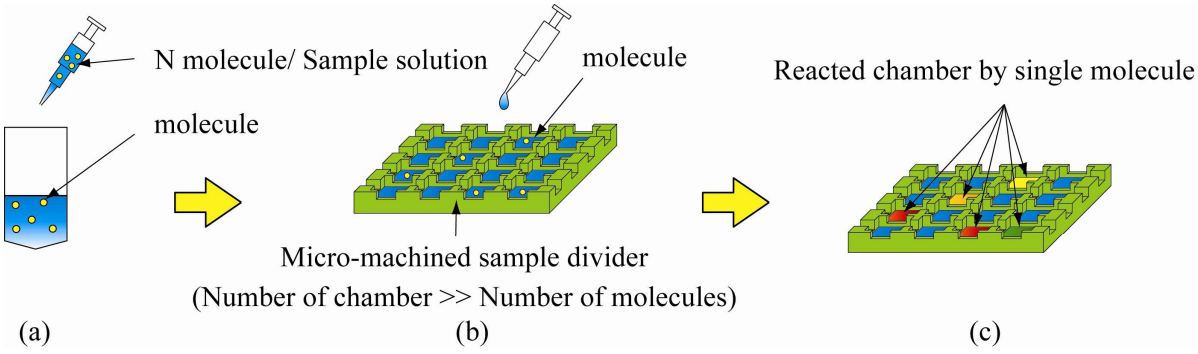


Figure 1: Concept of sample divider for biochemical reaction analysis based on single molecule.

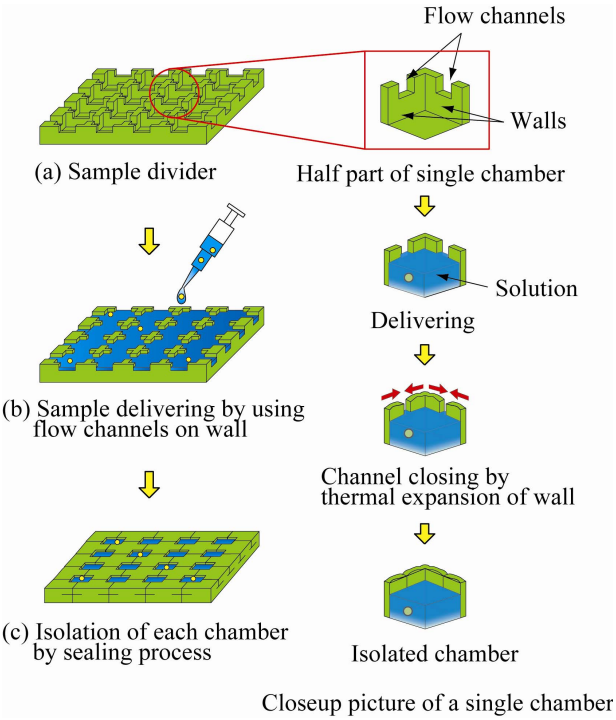


Figure 2: Operation principle of sample divider

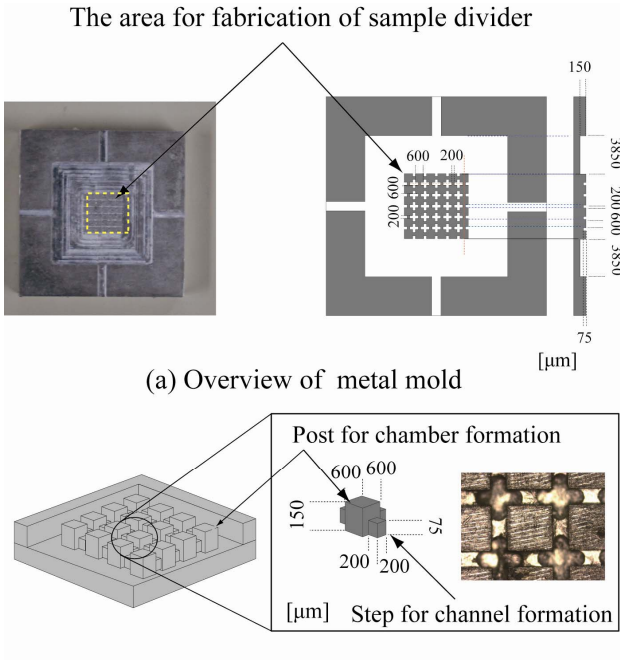


Figure 3: Fabricated metal mold by conventional machining process

(1) A single chamber unit in the divider consists of the four walls with liquid connection flow channels, as shown in Fig. 2. Therefore, the delivered solution is uniformly divided to all chambers by passing through the channel. The molecules are stochastically distributed to the chambers during the delivering process, and the number of the molecule at the most of the chambers is expected to become one or nothing, because of the huge number of chambers (number of chamber is enough larger than that of sample).

(2) The channel is sealed at once by using the thermal expansion of the walls (Fig. 2(c)). A composite of a PDMS and an expancel, is used as a wall material, to effectively close the channels by heat.

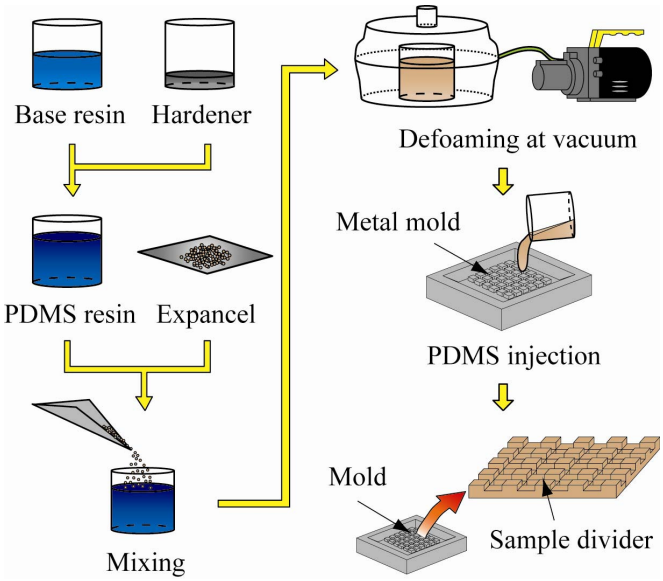


Figure 4: Fabrication process of sample divider

3. FABRICATION

We used the composite of a PDMS and an expancel as a material, and fabricated the sample divider structure by applying the mold process. The both fabrication process of a metal mold and the divider are as follows.

Metal mold

We used an aluminum plate as a substrate, and made a mold structure on its surface by using conventional mechanical machining process. The overview of the fabricated metal mold is shown in Fig. 3(a). The arrayed 6 x 6 unit structures were formed in the center section area of the plate for the following PDMS chamber fabrication. The detail configuration and the dimension of each unit mold structure is shown in Fig. 3(b). It consists of a center post and four steps. The post and steps were used as the chamber and the flow channel formations, respectively, in the following PDMS molding. The width and height of the post were 600 μm and 150 μm, respectively. The width and height of the single step were 200 μm and 75 μm, respectively. The pitch of the post was 800 μm.

Divider fabrication

We fabricated the sample divider by using the composite of PDMS and the expancel, as shown in Fig. 4. We used a commercially available expancel, supplied by Japan Fillite Co., Ltd. The expancel was the small plastic sphere and its volume becomes more than 60-times larger than that of the original by

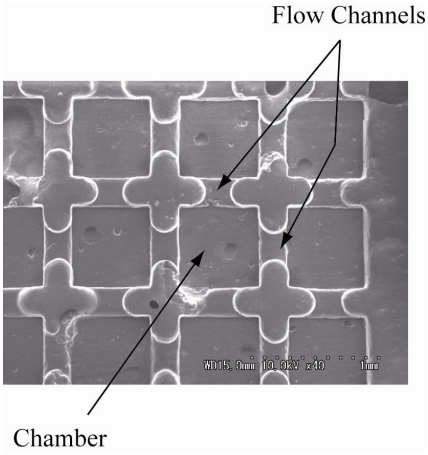
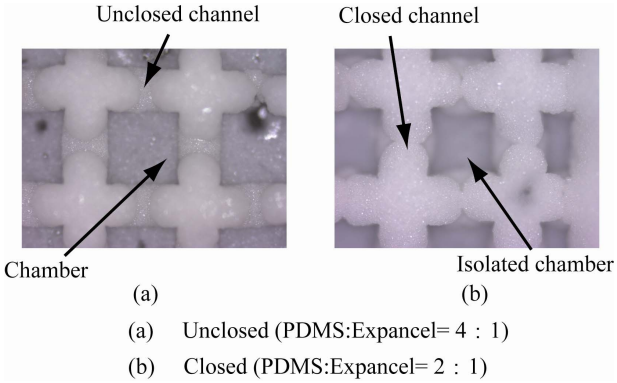


Figure 5: SEM photograph of fabricated divider



(a) Unclosed (PDMS:Expancel= 4 : 1)

(b) Closed (PDMS:Expancel= 2 : 1)

Figure 6: Unclosed and closed chambers by thermal expansion

heat. It was sometimes used as an actuation method for handling the liquid in Micro-TAS [8-10]. We added the expancel to PDMS solution at the constant ratio. After mixing them, we did the defoaming at vacuum. The composite of PDMS and expancel was poured on to the metal mold, and then cured at 60 degree-C. The sample divider was obtained by peel it off from the metal mold. The scanning electron microscope image of the fabricated divider is shown in Fig. 5.

4. EXPERIMENT

We investigated the PDMS and the expancel mixture fraction dependency, and effect of the heating time, on the channel closing performance.

Mixture fraction dependency

We at first investigated the mixture fraction between the expancel to the PDMS resin to close the channels effectively. The volume ratio between the expancel and PDMS was from 1:2 to 1:4. We heated the fabricated PDMS divider at 100 degree-C to close the channels. We used an optical microscope for the observation. The closing performances done by using the different mixture fractions are shown in Fig. 6. The walls formed at the volume ratio of 1:4 could not completely close the flow

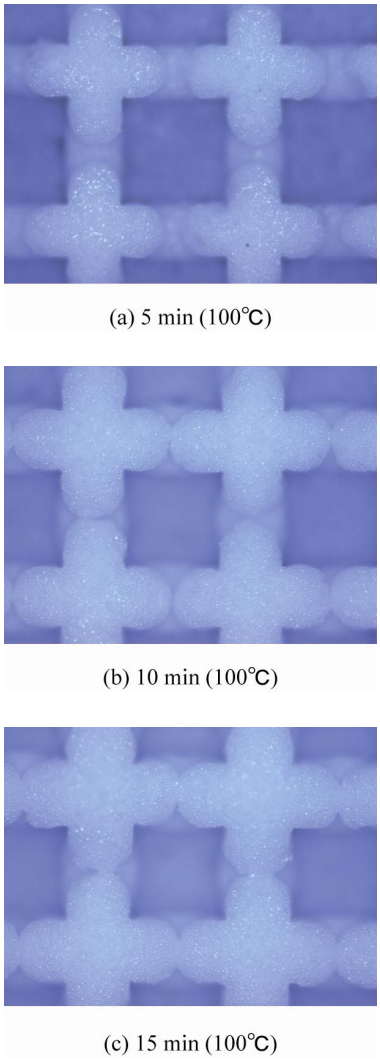


Figure 7: Difference of expansion by heating time

channels by the heat, as shown in Fig. 6(a). On the other hand, the ratio of 1:2 could close the channels successfully. We therefore used the volume ratio of 1:2, as an optimum value, in the following experiments.

Effect of heating time

We then evaluated the effect of the heating-time period on the channel closing performance. The results obtained at different heating-time periods are shown in Fig. 7. The walls could not close the channels when the time was 5 minutes, as shown in Fig. 7(a). They became the contact at 10 minutes (Fig. 7(b)), and finally completely closed the channels when the time period lengthened up to 15 minutes (Fig. 7(c)).

Liquid division

We experimentally evaluated the liquid dividing performance on the device. We used red colored water solution for clearly confirming the solution division. We at first dropped the solution on the sample divider, and then heated it at 100 degree-C for 15 minutes to close the channels. As shown in Fig. 8, the solution was successfully divided into small amount of it

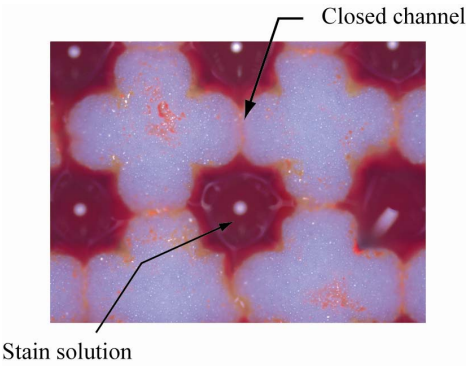


Figure 8: Appearance of chambers after optimizing

on the chip. From the result, we confirmed that the developed divider is able to divide the solution. However, the heating-time period of 15 minutes for the closing the flow channels are too long to prevent the evaporation. To overcome this problem, we are now thinking the two methods. One is the flow channel narrowing by using SU-8 mold structure, and the other is local heating of the flow channels.

5. CONCLUSION

We proposed novel type of a sample divider, which can easily divide the sample solution into multiple small amount of it on a chip, for single molecule analysis. The obtained results are as follows.

(1) We used the composite of a PDMS and an expancel as a material, and developed the fabrication process for the sample divider structure by applying the mold process.
(2) We investigated the PDMS and the expancel mixture fraction dependency, and effect of the heating time, on the channel closing performance. The flow channels were successfully closed when volume ratio between the expancel and PDMS solution was 1:2 and the heating time was 15 minutes.

From these results, we concluded that the proposed device is useful as the sample divider, if we optimally designed.

6. ACKNOWLEDGEMENTS

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