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RNA Trap using Microfluidic Chip with Taper Shaped Channel

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1. Introduction

No researcher who is into gene expressions can avoid the extraction of total RNA from a cell or tissue which is troublesome due to its laborious and precautionary protocol. On this research, we discovered a phenomenon of accumulation of charged biomolecules such as DNA or RNA near the constricted position of a microfluidic chip with taper shaped channel when both hydro pressure and electric field are applied in opposite directions. Making use of this phenomenon, we already succeeded in trapping of T4 DNA [1], however, RNA has not been able to trap so far, unlike huge and uniformly double stranded DNA molecules, RNAs are smaller in size with complicated conformation like blocks in lysed cell solution. By mild denaturation which changes RNA from these block-like structures into chain-like structures, we succeeded in trapping RNA using the above-mentioned chip device. This technique is expected to establish easy and practical device as a direct total RNA extraction tool from living cells or tissues.

2. Experiment

Fig. 1 shows the micro channel pattern, which is made of polydimethylsiloxane (PDMS)[2] using a conventional photolithography technique with SU-8 thick resist molding. The channel is 15 mm long, 100 μm wide, 10 μm deep, with a symmetrical taper shape having 10 μm wide, located at the center of the channel. Fig. 2 shows the experimental setup.

The total RNA from K562 cell (Human chronic myelogenous leukemia) was purchased from Maxim Biotech, Inc., and after mild denaturation by formaldehyde [3], it was stained with YOYO1 [4], an intercalating dye. The final sample solution was adjusted with the TBE buffer to prevent electroosmotic flow. The experiment began by addition of sample to the reservoir, and setting the pressure to flow the sample solution into the channel, changing the applied voltage between both the electrodes of the sample and the waste reservoir, step by step. This results in RNA trapping at the tapered region of channel,which is observed under a fluorescence microscope.

3. Results and Discussion

Fig.3 shows the examples of RNA trapping patterns, and Fig. 4 shows the conditions established for RNA trap. There are two kinds of RNA trap patterns observed. Pattern A has two trapping points at the both sides of the narrowest channel, that is, at relatively lower velocity of flow. On the other hand, pattern B has one central trapping stream line, that is, at the highest velocity of flow. The pattern A appears when electric field is weak, and the pattern B appears when applied electric field is rather strong. These facts are also shown in Fig. 4 where left sided area of the dotted line represents pattern A, and the right side represents pattern B, at each pressure, with increasing applied voltage. The pattern A always appears at the early stage of RNA trapping, and the transition of pattern A to the pattern B never fails to be observed. This transition is similar to one in DNA case [5]. Fig. 4 also shows the RNA trapping region represented by the bold solid lines. But, due to the restriction of the pressure proof of the chip and the maximum applied voltage of the power supply, in this experiment, the higher range over 20 kPa in pressure and 500 V in voltage could not be tested. Therefore, as shown by arrows, the area enclosed by the two extended line a-a and line b-b can be also considered as the RNA trapping condition.

4. Conclusion

RNA is successfully trapped by micro fluidic taper channel and wide RNA trap conditions were established. This result shows the realization of a on-chip RNA extraction is possible. The transition phenomenon of RNA trap pattern will be helpful to analyse the mechanism of the trapping phenomenon in a taper shaped channel.

Acknowledgements

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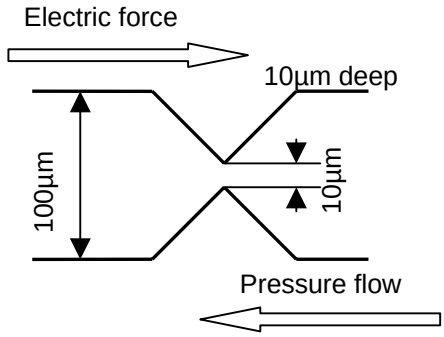

Fig. 1 Channel pattern for trap with a symmetrical taper and the cone angle of which is 90˚. The inner wall of the channel is coated with polyvinylprrolidone (PVP) so as to prevent electroosmotic flow.

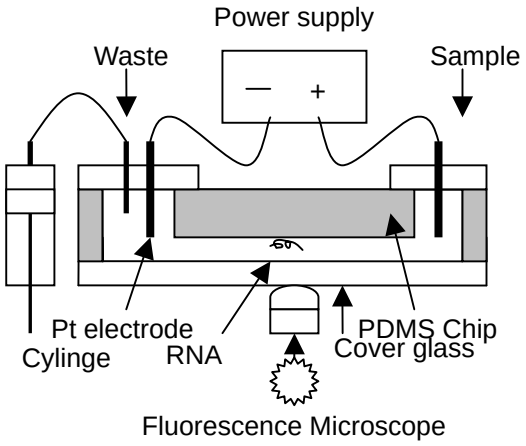

Fig. 2 Experimental setup. In this experiment , by pulling out the cylinge piston , the waste reservoir is negatively pressurized, so the sample solution including RNA flows towards the waste one. On the other hand, the nagative charged RNA is pulled towards positively charged Pt electrode.

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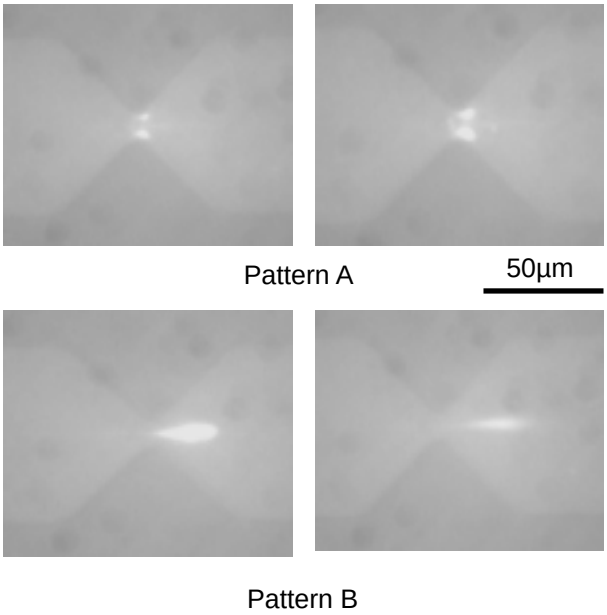

Fig. 3 Two types of RNA trapping patterns. Pattern A and pattern B are shown for the two typical examples.

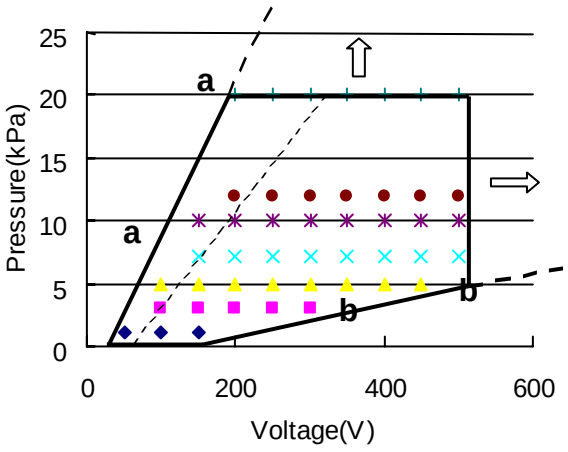

Fig. 4 Conditions of RNA trap where RNA trapping could be observed at the given voltage and pressure.