PAIRING BEADS WITH A MEANDER-SHAPED DYNAMIC MICROARRAY DEVICE

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ABSTRACT

In this study, we have developed a meander-shaped dynamic microfluidic technology that allows us to pair two different types of microbeads in a trapping site. In our device, we have successfully trapped different types of sample in one trapping site, and constructed an array of paired beads of different type such as polystyrene beads or hydrogel beads made of agarose, collagen or alginate. We found that this meander-shaped dynamic microfluidic technology is applicable for the observation of interactions between the paired beads such as molecular diffusion.

KEYWORDS: Dynamic Microarray, Pairing beads, Hydrogel bead

INTRODUCTION

Microarrays have been mostly applied in chemical analysis, diagnostics and drug screening. Recently, a dynamic microfluidic system in which bio-molecules and chemicals are immobilized onto mobile substrates such as microbeads, has aroused increasing interest in biomedical research and high-throughput assay applications. Some groups have reported dynamic microarray systems containing a dense array of weir-based passive hydrodynamic sample traps[1-2]. These devices can isolate samples at each trap quickly and easily in a purely hydrodynamic fashion. Due to random trapping, however, not all of the samples are successfully trapped. Recently, our group has proposed a meander-shaped dynamic microfluidic device that can transport and immobilize beads, infuse reagents, enable reactions to be observed, and enable selected beads to be retrieved[3-4]. Using this method, it is possible to observe single microbeads and to reduce sample volume since all of the introduced beads can be trapped at each site sequentially.

The next challenge in the modification of the dynamic microarray device requires the development of a system to enable two different types of sample to be properly trapped at each trapping site. The ability to make micro-objects come into contact with each other has attracted enormous interest for a wide variety of applications such as the analysis of cell-cell interactions and cell fusion. As has been reported[2], the weir-based hydrodynamic device has successfully enabled micro-sized objects to be paired at each site by using a 3-step loading protocol. On the other hand, in meander-shaped dynamic array devices, few efforts have been made to fabricate an array of more than two different types of sample.

In this study, we thus present an improved meander-shaped dynamic microfluidic device to trap and pair different types of microbeads. Since the device allows the sequential traps, it will be useful for pairing expensive or rare samples in an array without losing them. We demonstrate that different types of beads made from polystyrene or hydrogel can be paired at the traps, and show that interactions between paired hydrogel beads such as molecular diffusion can be detected.

THEORY

Fig. 1 shows schematic images of our microfluidic flow-through device, which consists of two parts: a meander-shaped by-pass channel and a trapping channel with a hydrodynamic trapping site along the by-pass channel. The microchannels are designed such that: (i) when a trapping site is empty, the trapping channel has a lower flow resistance than that of the by-pass channel and beads flow into the trapping stream and subsequently into the trap; (ii) the trapped bead acts as a plug, increasing the flow resistance along the trapping channel drastically; and (iii) the main flow redirects to the by-pass channel and subsequent beads will flow into the by-pass stream, by-passing the filled trapping site. Here, we form the line symmetrical by-pass and trapping channels, and thus two trapped beads are able to come into contact in one trapping site.

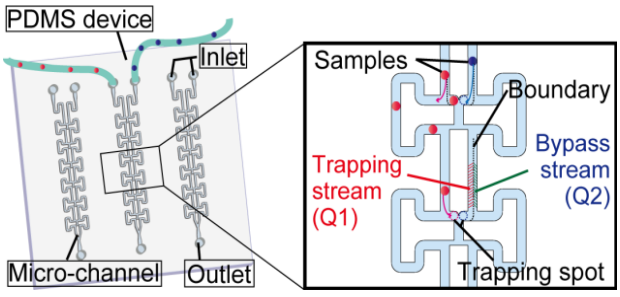

Figure 1: Schematic images of a microfluidic device that utilizes meander-shaped dynamic microfluidic technology to allow microbeads to be transported and immobilized during microfluidic experiments, and to allow different types of microbeads to be paired.

EXPERIMENTAL

Microfluidic channels were fabricated in poly(dimethylsiloxane) (PDMS) using conventional soft lithography techniques. In order to introduce the microbead solution to the PDMS device, we used syringe pumps and syringes. A 3CCD camera mounted on an inverted microscope was used to image the process. The diffusion performed in this study was observed under a fluorescence microscope. Chambers were kept in the dark during experimentation, and the fluo-

rescence intensity of calcein was recorded at 33 milli-second intervals. The fluorescence intensity in selected regions within each hydrogel bead was determined using ImageJ software and plotted as a function of time.

We prepared three kinds of microbeads: (a) 100 μm-sized polystyrene beads; (b) collagen hydrogel beads; and (c) alginate hydrogel beads. We prepared a polystyrene bead suspension by adding beads to 1 ml of ultrapure water with 1% of Tween 20 added as surfactant. An axisymmetric flow-focusing device was employed for producing monodisperse droplets and hydrogel beads after gelation[5]. Collagen hydrogel beads were produced by gelation of collagen solution by following a previously reported fabrication process[6]. We put two types of cells, NIH3T3 fibroblast cells visualized green and Human hepatoblastoma HepG2 cells visualized red, around the collagen beads and cultured the cells by incubating them overnight on an orbital shaker in order to seed cells onto the collagen beads. Then, we introduced the cell-coated collagen beads into the microchannels of the microfluidic device and trapped them separately in the trapping sites. Alginate gel beads were produced by gelation of 1.5% sodium alginate solution. The alginate hydrogel beads were introduced into the microchannels as microreactors encapsulating fluorescent dye; in this study, we selected calcein. Hydrogel beads were suspended in mineral oil with surfactants. In the experiment to observe the diffusion between beads, we put a pillar at the beginning of trapping channels; otherwise the trapped hydrogel beads tend to deform and pass through the trapping site when at the high flow rate.

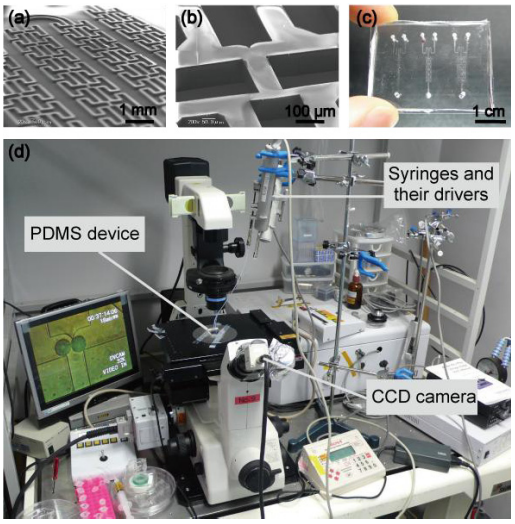

Figure 2: (a), (b) SEM images of SU-8 mold. (c) An image of cover glass bonded with PDMS slab with microchannels. (d) Experimental setup.

RESULTS AND DISCUSSION

The trapping channel displayed a lower flow resistance than the by-pass channel until a bead was trapped, thereby leading to a sequential filling of all down-stream trapping sites. We introduced 100 μm polystyrene beads labeled with different colors from the right and left inlets of the microfluidic device. The device captured and immobilized the first beads. Fig. 3 show the by-passing and trapping modes, respectively. By the change of flow resistance along the trapping channel, a trapped bead acts as a plug causes the following beads to flow into the by-pass channels and toward the down-stream trapping sites. Fig. 4(a-b) shows trapped polystyrene beads in the trapping sites.

We also tested the pairing of cell-coated collagen hydrogel beads. Fig. 4(c-f) shows two different types of hydrogel beads paired in a trapping site. In spite of their elasticity, hydrogel beads were carried into the trapping site without passing through it, and the beads were then brought into contact with each other using the fluid velocity in the same way as polystyrene bead trapping. It was observed that green stained NIH3T3 cells and red stained HepG2 cells are immobilized adjacent to each other. It is known that the co-culture of 3T3 with HepG2 enhances the secretion of albumin from HepG2, and that these are used to assess the activation of cells by cell-cell interaction. We expect that our microfluidic device will be used as a tool to quantify cell-cell communications in the future.

We observed that calcein can diffuse from one bead to another by evaluating the changing fluorescence intensity of the calcein in paired beads. Briefly, hydrogel beads were suspended in mineral oil so that calcein molecules are unable to diffuse into mineral oil media but diffuse into another bead when they come into contact. In our device, we paired

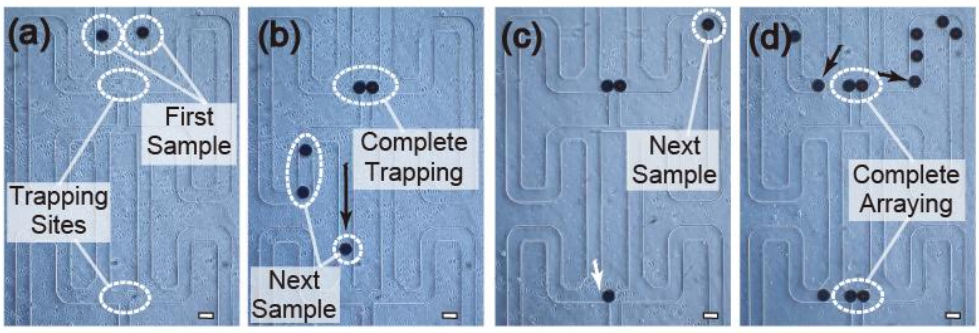

Figure 3: Images showing how beads are trapped in microfluidic devices. (a-b) When the trapping sites are empty, the first beads through the trapping channels are carried into them. (c-d) When the trapping sites are filled by the first bead, all following beads are carried along the by-pass channel, by-passing the trapping sites. This design allows for one bead to be trapped per trapping site.

hydrogel beads with and without calcein in the trapping sites as shown in Fig. 5(a). Due to the concentration gradient between them, the calcein molecules started to diffuse out through the nanopores of the hydrogel beads.

Fig. 5 (b) shows the change of fluorescence intensity at the center of the right and left beads. In this graph, no reaction could be observed between the two beads just after they came into contact with each other. However, after increasing the flow velocity, we detected the diffusion of calcein from one bead to the other. We observed the diffusion start in more than 80% pairs of beads by the flow velocity, 100 nl/sec. This result can be explained as follows. A thin lipid layer would form between two beads due to the presence of surfactants mixed in the mineral oil ; in this experiments, we used lipid molecules (lecithin). This lipid layer might disturb the diffusion of the calcein. By increasing the flow velocity, on the other hand, the lipid layer would break and subsequently the two beads form a connection that promotes the diffusion of calcein from one bead to the other.

After the diffusion started, we found that it took calcein molecules more than 20 seconds to diffuse out of the right hydrogel beads and fill the left hydrogel beads. Theoretically, the molecular diffusion in a solution is described as "$t = l^2/4D$", where t, l and D is diffusion time, diffusive region length and diffusion constant, respectively. Therefore, t can be estimated to be 8.3 seconds when l = 100 μm and $D \sim 30\times10^{-7}$ cm^2/s for a molecule with radius of approximately 1 nm[7]. In this case, the diffusion took about twice longer than that in the case of diffusion in a solution. This result is probably attributable to the reduction of diffusion constant for calcein in alginate hydrogel whose pore size is ranged 5 nm ~ 200 nm[8].

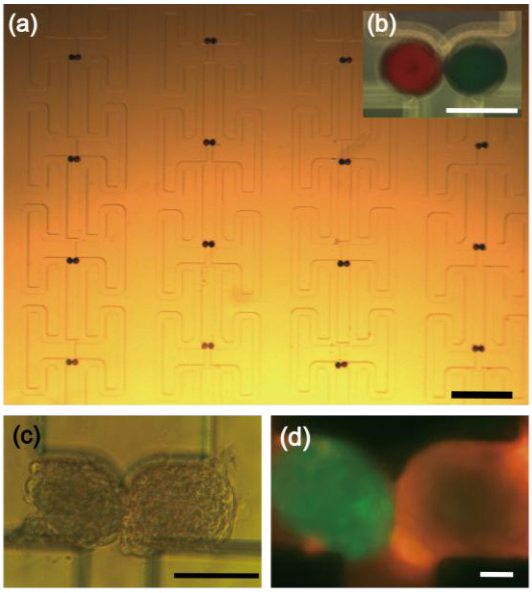

Figure 4: An optical image of arrayed (a) 100 μm-sized polystyrene beads, (b) paired 100 μm-sized beads. (c) Images of collagen hydrogel beads and (d) fluorescence images of collagen hydrogel beads with stained cells. The scale bars are (a) 1 mm, (b)–(c) 100 μm and (d) 10 μm.

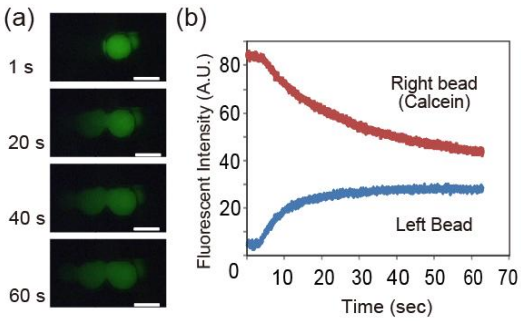

Figure 5: Fluorescence images of paired alginate hydrogel beads. (a) The diffusion of calcein from the right bead to the left bead. (b) Fluorescence intensity vs. time graph for the molecular diffusion of calcein from the right bead to the left bead. The scale bars are 100 μm.

CONCLUSION

We have developed a meander-shaped dynamic microfluidic technology that allows microbeads to be transported and immobilized during microfluidic experiments, and allows different types of microbeads to be paired. This simple microfluidic device is robust, easy to fabricate and operate compared to other recent work reported on bead trapping arrays. This device is also applicable to the observation of interactions between paired beads. We thus expect that our device will play an important role in the continuous observation of cell-cell interactions or molecular diffusion.

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