ARRAYING AND SHUFFLING TRIPLE MICROBEADS WITH DYNAMIC MICROARRAY DEVICE

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ABSTRACT

We present a highly parallel microfluidic approach for contacting three different microbeads in an array and changing their contacting mode (e.g. ABC→CAB→BCA→ABC) (Fig. 1). For arraying the beads, we add microvalves in the previously reported dynamic microarray [1] to control the fluidic resistance. Moreover we can shuffle the arrayed beads just by applying a reverse flow. This method would enable us to achieve not only bead-based chemical reactions using triple hetero microbeads at one time, but also temporal and spatial control of reactions between the beads by the control of contacting mode.

KEYWORDS: Dynamic microarray, Bead-based analysis, Microfluidic device, Cell pairing, Microbead

INTRODUCTION

The advancement of microfluidic platforms for bead-based biochemical analysis impacts a broad range of biochemical and biomedical fields, including genomics, proteomics, drug discovery, and infectious disease diagnostics [2]. System miniaturization results in a variety of benefits, low reagent volumes, and rapid diffusion times. As a result, there have been many kinds of microarray for bead-based chemical reactions [3]. Recently, the device for paired bead-based reactions has been developed in order to analyze reactions between different types of beads [4]. However, the device which enables us to achieve triple-hetero-bead-based reactions has not been reported. Triple-hetero-bead-based reaction is, for example, desired for the detailed and further analysis of cell-cell communication and cell differentiation using triple types of cells (encapsulated in or attached on hydrogel beads). Compared with paired microbeads, contacting triple hetero microbeads have a notable characteristic; multiple contacting modes (ABC, CAB, BCA). Therefore, control of the contacting mode is significant for control of chemical reactions. For example, it would be possible to rearrange the cells after arraying and stop/proceed chemical reactions. Here, we developed a dynamic microarray device for contacting triple hetero beads and controlling contacting modes dynamically (Fig. 1).

PRINCIPLE

In our device, units of microfluidic channel including trapping spots and bypass channel (Fig. 2) are in an array. The dimension of the units is calculated using the Darcy-Weisbach equation so...
that, when the trapping spot is vacant, beads are trapped into the trapping spot, and when a bead has already trapped, the next bead goes along bypass channel. In each unit, a valve is located beside a trapping spot. Therefore, we can change a fluidic resistance of the trapping spot so that only one bead is trapped into the trapping spot at one time.

**EXPERIMENTAL**

The dynamic micro array device was made from Polydimethylsiloxane (PDMS) and Glass substrate, and fabricated by standard soft-lithography. Trapping spots were arrayed along a microfluidic channel. The height of the device for arraying of 100 µm-beads was 120 µm.

The device has microvalves (dead-end channel) to control the fluidic resistance. The microvalves were filled with deionized water. The channel of microvalves were connected to a microsyringe: When we infused/withdrew water into the valves, they were switched on/off (expanded/restituted), respectively.

In the device, the solution was infused into each microfluidic channel by a syringe pump (Instech Laboratories, pico plus) via a teflon tube and a syringe. The flow velocity was ranged from 3 to 7 µl/min. The fluidic resistance does not depend on the flow rate theoretically: Basically, any velocity can be applied for trapping or shuffling microbeads.

Polystyrene microbeads were purchased from Microparticles GmbH (ϕ=100 µm, Berlin, Germany). Tween 20 as surfactant was purchased from Kanto Chemical (Tokyo, Japan). When we used polystyrene beads, we dispersed them into the de-ionized water (Milli-Q) containing 2 w% of Tween 20 in order to avoid aggregation of the beads.

The alginate hydrogel beads were made from sodium alginate and CaCl₂ aqueous solution. The sodium alginate was purchased from Wako Pure Chemicals Industries (Osaka, Japan). The CaCl₂ was purchased from Kanto Chemicals (Tokyo, Japan). The diameter of the alginate hydrogel beads in our experiments were 100 µm. We made fluorescent hydrogel microbeads by addition of green fluorescent nanobeads (diameters were 200 nm, Invitrogen, USA) inside hydrogel microbeads. These hydrogel beads were dispersed into the pure water.

**RESULTS AND DISCUSSION**

Contacting of triple hetero microbeads was achieved as follow in Figure 3. First, blue beads were infused into the microchannel by the forward flow with the valves switched on (Fig. 3a), and then the blue beads were trapped into the trapping spots (Fig. 3b). After switching off the valves, the blue beads were trapped into the origina...
beads went inside trapping spot (Fig. 3c). Second, red beads were trapped in the same way as the blue ones (Fig. 3d). After switching off the valves, the red beads went inside the trapping spots (Fig. 3e). Finally, green beads were infused and trapped, resulting in contact of triple hetero microbeads (Fig. 3f).

Shuffling triple hetero microbeads in order to change contacting mode was achieved as follows (Fig. 4). After arraying triple hetero microbeads (Fig. 4a), the back-flow was applied, and then microbeads went out from the trapping spots to the channel (Fig. 4b). Then, the green beads were trapped into the next trapping spots, and the red and blue beads went along the bypass channel (Fig. 4c). Flow direction was redirected to forward, and the green beads went out from the trapping spots to the channel resulting in rearranging the microbeads in contact (Fig. 4d). Finally, these triple microbeads were trapped into the original trapping spots (Fig. 4e). As a result, only by operation of two-way flow, we successfully changed the three types of contacting modes: ABC→CAB (from blue, red, green to green, blue, red) (Fig. 4f). By the same operation, the other contacting modes (CAB→BCA→ABC) were achieved. In addition, our system can be applied for hydrogel beads in which some materials such as cells and chemicals can be included. In this work, we demonstrated trapping and shuffling hydrogel beads by use of alginate hydrogel beads (Fig. 5). Figure 6(a-c) show three types of contacting modes.

CONCLUSION

We firstly succeeded in contacting triple hetero microbeads using a dynamic microarray device and controlling contacting mode. In addition, we indicated that our system was also applicable for hydrogel beads. We envision to observe chemical reactions among triple hydrogel beads, for example, molecular diffusion and molecular exchange among triple hetero cells.

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