MICROFLUIDIC PARTICLE CLUSTERING DEVICE FOR CROSS-CONTAMINATION-FREE MULTIPLEX ANALYSIS

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ABSTRACT

This paper describes a dynamic microarray device that facilitates a particle-based multiplex analysis by co-locating multiple, hetero particles at a designated reaction chamber array. The device allows parallel monitoring of multiple reactions among clustered particles. A high-density particle cluster array was formed by utilizing flow-induced deformability of a polydimethylsiloxane (PDMS) membrane that is caused by applied hydrodynamic pressure. By incorporating immiscible oil isolation, particles clusters were isolated from the outer environment, which enabled a cross-contamination-free analysis. We monitored the interactions among clustered hetero particles, without any cross-contamination, by co-locating polystyrene beads and chemical-laden hydrogels within arrayed reaction chambers and isolating them using immiscible oil.

INTRODUCTION

Microfluidic-based platforms incorporating microparticles have attracted great attention in recent years because of their multifunctionality [1]. Co-locating multiple particles of different types (e.g., cells, beads, and hydrogels) in spatial confinements facilitates the study of interactions between the particles because each particle can behave as an individual microcarrier containing chemicals or biomolecules. Recently, numerous strategies have been developed to achieve pairing or clustering of particles, including microwell [2] and microfluidic cell trap arrays [3], and valve including microfluidics [4]. However, these approaches have some limitations. For microwell arrays, different types of particles undergo random distribution and pairing rather than controllable particle pairing or clustering. Cell trap arrays can be applied only to pair or cluster deformable cells or droplets, not rigid particles. Although valve including microfluidics can manipulate various types of different particles, rigid or deformable, additional equipment is required for valve operation.

In addition, for these array-based approaches, cross-contamination between neighboring array elements is a potential obstacle for conducting multiplex analysis. Therefore, to be utilized as an effective individual reaction chamber, each array element containing multiple particles should be isolated from the other ones.

In this paper, we present a simple method to achieve particle cluster arrays and parallel monitoring of multiple reactions among clustered particles in a single chip without any potential for cross-contamination. By exploiting a flow-induced deformability of the membrane structure,

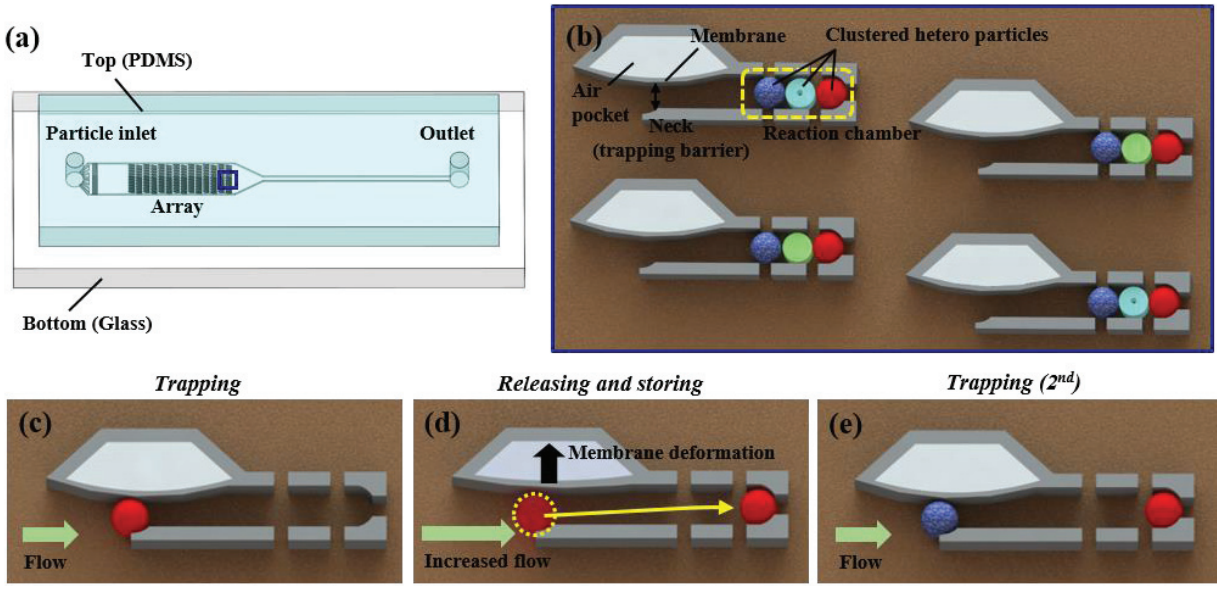

Figure 1: (a) Schematic illustration of the dynamic microarray device. (b) An enlarged schematic view of an array, showing the composition of an array element and clustered hetero particles. (c-e) Operation sequence for particle clustering. Introduced particles are trapped at the neck under pressure-driven flow (Trapping). Trapped particles are released and enter the chamber by flexible membrane deformation under a briefly increased flow rate (Releasing and storing). Restored membrane captures another incoming particle (2nd trapping). Multiple particles can be clustered in a specific order by repeating this process. Clustered particles are secured in the chamber under a continuous flow because of the narrow constriction.

different types of particles (rigid or deformable) were clustered in a specific, desired order. Additionally, the incorporation of immiscible oil easily enabled the isolation of each element in the particle cluster array.

MATERIALS AND METHODS

Device design and operation mechanism

The polydimethylsiloxane (PDMS) microfluidic device consists of a particle inlet, an array for clustering particles, and an outlet (Figure 1a). The array element is composed of an air pocket, a neck, a membrane (~5 μm thickness), and a reaction chamber (Figure 1b). Clustering of multiple hetero particles within the chamber in a specific, desired order can be facilitated by repeating a "trapping-releasing-and-storing" sequence (Figure 1c-e) that can be achieved by utilizing the flow-induced deformation of the flexible PDMS membrane without applying external force. Introduced particles are captured at the neck under a moderate flow (Figure 1c). Captured particles can be released and then let into the chamber through the membrane deformation under a temporarily increased flow rate (widening of trapping gap) (Figure 1d). Subsequently, stopping the increased flow restores the original shape of the deformed membrane, allowing the capturing of another particle (Figure 1e). As we employ the deformability of the membrane (not that of the particles), different particle types (e.g., cells, hydrogels, droplets, beads) can be manipulated, regardless of their deformability.

Fabrication

The particle clustering device and the mold for hydrogel fabrication were fabricated with polydimethylsiloxane (PDMS) using a standard soft lithography. Two different silicon masters were prepared by patterning negative photoresist for the particle cluster device and hydrogel micromold (thicknesses equal to 30 μm and 24 μm, respectively). For the particle clustering device, the prepolymer and curing agent (12:1 w/w ratio, Sylgard 184, Dow Corning Inc.) were poured into the master, cured, and peeled off from the master and then bonded to a glass substrate by O_2 plasma treatment after punching the inlet and outlet holes. The micromold for hydrogel fabrication was made using PDMS (10:1 w/w ratio) and was replicated from the silicon master; it consisted of hole arrays with a diameter of 25 μm.

Particle preparation

In this study, four different types of beads (commercially available) and custom-made hydrogel beads were used. Bare polystyrene (PS) beads (~25 μm diameter) were purchased from Sigma-Aldrich. Blue-colored PS beads (~26 μm diameter) and red-fluorescence PS beads (~25 μm diameter, excitation/emission = 530 nm/607 nm) were purchased from Microparticles GmbH. Streptavidin-coated PS beads (~25 μm diameter) were purchased from Spherotech. The concentration of all PS beads was 2×10^6 beads per mL when mixed with phosphate-buffered saline (PBS) solution containing 0.4% (v/v) of Tween 20. Poly(ethylene glycol)diacrylate (PEGDA, Mn = 700), Tween 20, 2,2-Diethoxyacetophenone (DEAP), and Dulbecco's phosphate buffered saline (DPBS) were purchased from Sigma-Aldrich. Cy3-labelled biotin molecule was purchased from Bioneer. Phosphate-buffered saline (PBS) solution was purchased from Gibco. Hydrogel beads were fabricated by UV photopolymerization (365 nm for 3 min) using the PDMS micromold. Finally, the composition of the hydrogel prepolymer solution was 50% (v/v) PEGDA containing 1.5% (w/v) DEAP and 50% (v/v) Cy3-labelled biotin in DPBS solution or 50% (v/v) DPBS solution.

Experimental setup

The experimental setup consisted of a microfluidic device, a custom-built vacuum pumping system, and a syringe pump (Fusion Touch 200, Chemyx Inc.). Microscopic images were acquired with an inverted microscope (IX 73, Olympus) and recorded using a charge

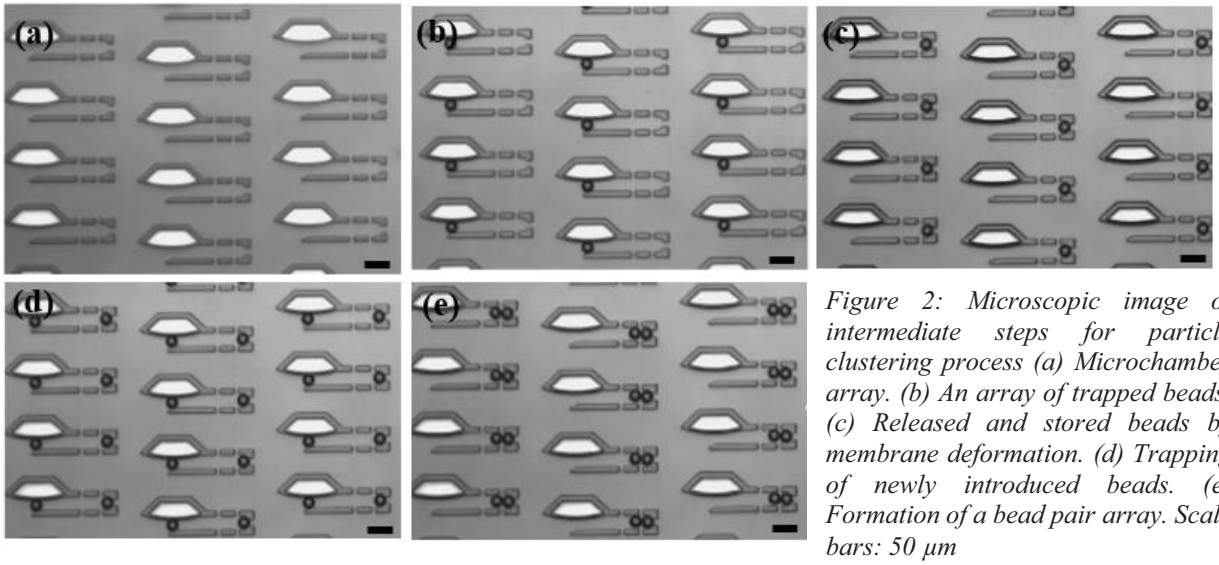

Figure 2: Microscopic image of intermediate steps for particle clustering process (a) Microchamber array. (b) An array of trapped beads. (c) Released and stored beads by membrane deformation. (d) Trapping of newly introduced beads. (e) Formation of a bead pair array. Scale bars: 50 μm

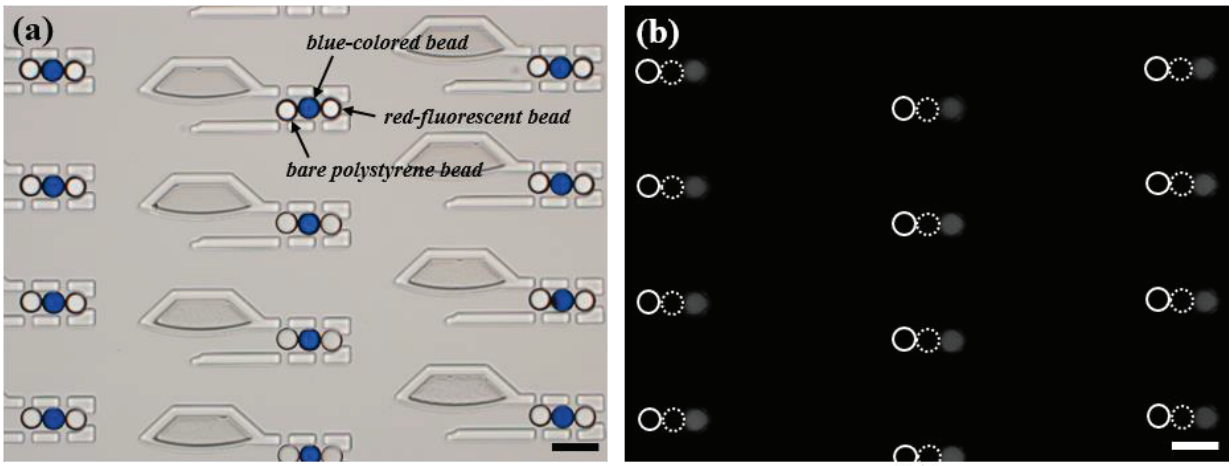

Figure 3: (a) Bright-field images of clustered hetero particles. (b) Fluorescence images of clustered triple hetero particles. Particle cluster array was constructed by repeating the "trapping-releasing-and-storing" process. Scale bars: 50 μm

coupled device (CCD) camera (DP80, Olympus). SOLA light engine (SM 365, lumencor) was used as the light source for UV photopolymerization of the hydrogel and fluorescence detection of particles.

RESULTS AND DISCUSSIONS

Particle clustering procedure

For a proof of concept, we used bare polystyrene beads and constructed an array of bead pairs using membrane deformation caused by hydrodynamic pressure change. The entire particle clustering was implemented using the multi-step "trapping-releasing-and-storing" technique (Figure 2). Initially, the polystyrene beads were introduced into microchannel when negative pressure was applied using a vacuum pump. The beads were trapped at the neck as they moved downstream. When the neck was occupied by beads, the streamline passing through the neck was diverted, and thus subsequent beads bypassed the chamber. After the chambers were occupied by beads (>90%), redundant beads were washed away at a flow rate of 50 μL/min (Figure 2b). Trapped beads were released from the neck and entered the reaction chamber by membrane deformation after increasing the flow rate (500 μL/min, ~ 5 s) (Figure 2c). Owing to the membrane's thickness, which is approximately 5 μm, the elastic membrane can deform under the imposed hydrodynamic pressure. The deformed membrane returned to its original shape instantly once the flow rate was decreased. Then, a second set of bead suspensions was introduced, trapped, and released in the same manner as the first bead "trapping-releasing-and-storing" process (Figure 2d-e). By repeating this process, multiple numbers of beads can be clustered in a specific, desired order.

Hetero triple particle clustering

By using the proposed method, clustered particles successfully formed an array (Figure 3). For the experiment, three types of polystyrene beads (bare, blue-colored, and red-fluorescent) were used. Initially, the red-fluorescent beads were introduced and trapped at the neck of the microchamber array. After releasing the red-fluorescent beads, the blue-colored beads and bare polystyrene beads were clustered in sequence by repeating the "trapping-releasing-and-storing" procedure.

Monitoring of interactions among clustered particles

We also demonstrated the monitoring of array-based multiple reactions between clustered hetero particles with oil isolation to prevent cross-contamination between array elements (Figure 4a-c). Streptavidin-coated polystyrene beads, PEGDA hydrogels, and bare polystyrene beads were clustered in the mentioned order; all the chambers were isolated from each other by infusing immiscible oil (FC-40). This oil isolation method is a simple and robust way to achieve chamber isolation [5].

As the encapsulated Cy3-labelled biotin molecules were released from the hydrogel over time and bound to adjacent streptavidin-coated beads, the fluorescence signal of streptavidin beads increased over time (solid arrow, Figure 4c). Meanwhile, streptavidin beads adjacent to the hydrogel beads without biotin-Cy3 conjugates showed no fluorescence signal change over time (dashed arrow, Figure 4c), which indicates that there was no cross-contamination between the reaction chambers. To investigate the effect of oil isolation, the cross-contamination between the neighboring reaction chambers was monitored in a single phase (i.e., non-isolated chamber) condition (Figure 4d-f). Released biotin-Cy3 molecules migrated to other reaction chambers by diffusion and then bound to the surface of the streptavidin coated beads located in the other reaction chambers (solid arrow, Figure 4f). These results indicate that the oil isolation was successful and multiple reactions among clustered particles can be analyzed without cross-contamination.

CONCLUSION

In this study, we demonstrated a particle-based multiplex analysis method with no cross-contamination. This

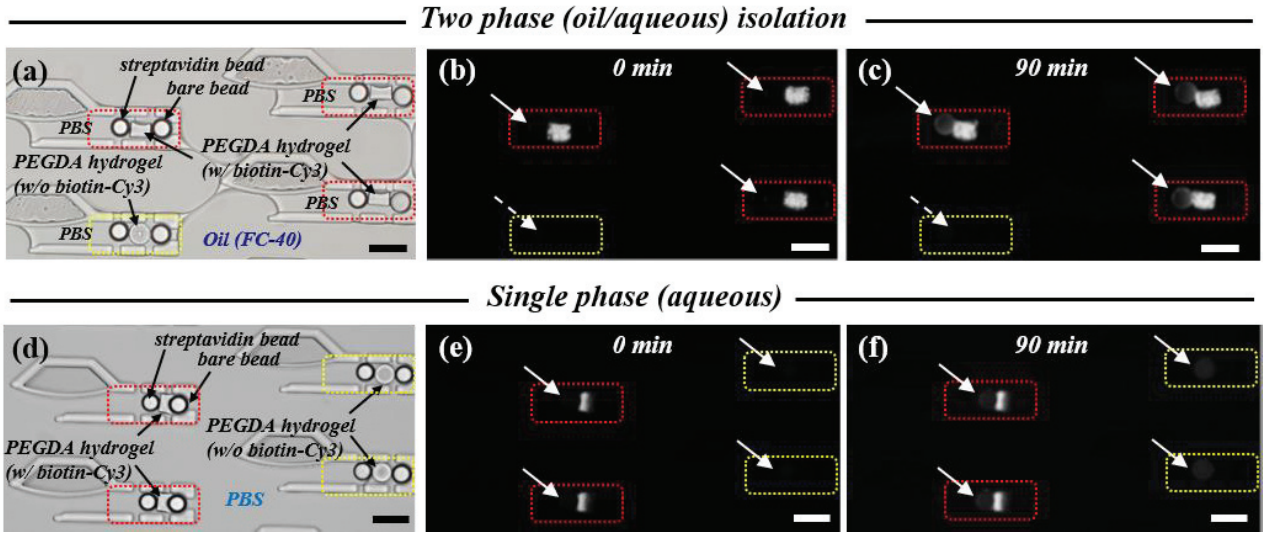

Figure 4: Microscopic images of clustered particles (bare polystyrene bead, streptavidin conjugated polystyrene bead, PEGDA hydrogel) showing the effect of oil isolation. Encapsulated Cy3-biotin molecules were released from PEGDA hydrogel and bound to the adjacent streptavidin conjugated bead. Fluorescence signal of streptavidin conjugated bead was observed to monitor the bead-based interaction. The experiment was conducted under two different conditions; (a) isolated and (d) non-isolated reaction chamber array. No cross-contamination was observed in the isolated chamber array (b-c) while cross-contamination was observed in the non-isolated chamber array (e-f). Scale bars: 50 µm.

study was facilitated by the construction of an array of particle clusters and the isolation of each cluster unit using immiscible oil. The entire particle clustering process was performed via the repetition of the "trapping-releasing-and-storing" process, which exploits the flow-induced deformability of the elastic PDMS membrane. We have formed an ordered clustering of hetero, multiple particles and monitored the interactions between the clustered particles over time without any trace of cross-contamination using oil isolation method. We believe that our device has the capability of a continuous and parallel monitoring of various interactions between multiple hetero particles (e.g., cell-bead or cell-hydrogel) in a confined microenvironment.

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REFERENCES

[1] S. Derveaux, B. G. Stubbe, K. Braeckmans, C. Roelant, K. Sato, J. Demeester and S. C. De Smedt, "Synergism between particle-based multiplexing and microfluidics technologies may bring diagnostics closer to the patient", *Anal Bioanal Chem*, vol. 391, pp. 2453-2467, 2008.

[2] I. Zaretsky, M. Polonsky, E. Shifrut, S. Reich-Zeliger, Y. Antebi, G. Aidelberg, N. Waysbort and N. Friedman, "Monitoring the dynamics of primary T cell activation and differentiation using long term live cell imaging in microwell arrays", *Lab Chip*, vol. 12, pp. 5007-5015, 2012.

[3] B. Dura, Y. Lin and J. Voldman, "Deformability-based microfluidic cell pairing and fusion", *Lab Chip*, vol. 14, pp. 2783-2790, 2014.

[4] T. Tonooka, T. Teshima and S. Takeuchi, "Clustering triple microbeads in a dynamic microarray for timing-controllable bead-based reactions", *Microfluid Nanofluid*, vol. 14, pp. 1039-1048, 2013.

[5] Y. –C. Chen, Y. –H. Cheng and E. Yoon, "CELL-NICHE-ON-CHIP: PAIRED SINGLE CELL CO-CULTURE PLATFORMS USING IMMISCIBLE LIQUID ISOLATION AND SEMI-PERMEABLE MEMBRANES", in *Digest Tech. Papers IEEE MEMS '14 Conference*, San Francisco, January 26-30, 2014, pp. 943-946.

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