論文の内容の要旨

"Dynamic Microarray Technology for Biochemical Applications"

（生化学応用のためのダイナミックマイクロアレイ技術）

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ABSTRACT

We present two achievements in this work: (i) the development of a dynamic micoarray platform by combining hydrodynamic with optical-based methods, and (ii) the preparation of monodisperse cell-encapsulating alginate hydrogel microbeads with coefficient of variation (C.V.) less than 5% by combining T-junction droplet formation with internal gelation method. Based on these results, we developed a gentle and easy to handle bead-based cell assay system.

1. INTRODUCTION

Researchers envisage using cell-based microsystems in the studies of pathological and physiological phenomena in cells, which will have enormous potential for cell-based diagnostic applications, drug testing and toxicology studies. However, this potential has yet to be fully realized due to the lack of reliable multi-functional platforms to transport and immobilize particles, infuse reagents, observe the reaction, and retrieve selected cells. Moreover, most of the proposed devices to date are not capable of handling both adherent and non-adherent cells.

Here, we developed a dynamic microarray technology that allowed us to achieve all these functions in a single integrated device through the combination of hydrodynamic and optical approaches. Hydrodynamic forces allow simultaneous transportation and immobilization of large number of particles, while optical-based microbubble technique for bead retrieval gives dexterity in handling individual particles without complicated circuitry. Also, we developed cell-encapsulating techniques in alginate hydrogel microbeadsby combining T-junction droplet formation with internal gelation method [1]. Development of this encapsulation technique serves three purposes: (i) Size variation of individual cells make them difficult to work with and encapsulation in monodisperse capsules solves this problem, making them compatible with our platform; (ii) Encapsulation allows us to work with both adhesion and non-adhesion cells; (iii) Alginate hydrogel protects fragile cells from mechanical stresses, facilitating manipulation.

2. DESIGN

μ-Fluidic trap and optical-based microbubble retrieval system (Trap-and-retrieval device)

The μ-Fluidic trap is made up of a square wave shaped channel superimposed onto a straight channel (Figure 1). The traps are narrowed regions along the straight channel. When the trap is empty, flow resistance along the straight channel is lower than that of the loop channel, and the main stream flows along the straight channel. A bead in the flow will be carried by the main stream into the trap (Trapping mode). Once the trap is filled, flow resistance is increased drastically along the straight channel, and the main flow is redirected along the loop channel.

When all the traps are occupied, subsequent particles are not able to enter occupied traps and will bypass the filled traps (Bypassing mode), following the main flow out of the device. Taking advantage of this characteristic, we can retrieve a trapped particle from the array by displacing it back into the main flow using microbubbles. Here, we propose a simple optical-based method to create microbubbles without any need for circuits and connections. Aluminum patterns, functioning as heaters, are located near the narrowed region of the μ-Fluidic traps (Figure 2). When we focus an Infra-red (IR) laser onto the aluminum pattern, localized heating results in bubble formation and the expanding bubble displaces the immobilized particle from the μ-Fluidic trap into the main flow. The displaced particle is then carried by the flow out of the device where it can be collected.

Micro T-junction device for producing alginate hydrogel microbeads

Uniformly-sized droplets are formed when sodium alginate solution containing insoluble calcium carbonate nanoparticles is introduced into the stream of oil at a T-junction (Figure 3). Lecithin and acetic acid dissolved in oil is then added downstream. The pH reduction releases Ca^{2+} from the insoluble calcium complex, causing gelation. Beads are subsequently collected in a microtube and centrifuged to separate them into aqueous solution. Using this device, cells can be easily encapsulated in the beads by simply adding cells to the sodium alginate solution.

3. FABRICATION

Soft lithography using elastomer PDMS (Sylgard® 184 Silicones Elastomer, Dow Corning) was chosen for micro fabrication technology of both the trap-and-release device and the micro T-junction device. Firstly, SU-8 mold was made on silicon wafer using standard lithography techniques. PDMS layer was then cast from SU-8 master mold. Access holes for inlets and outlet were punched on the PDMS slab. For the trap-and-release device, the PDMS slab was aligned and bonded with a cover glass patterned with aluminum. For the micro T-junction device, the PDMS slab was bonded to another PDMS slab prepared by curing PDMS in a Petri dish.

4. RESULTS AND DISCUSSIONS

Dynamic Microarray platform

We connected the μ-Fluidic Traps in series to create an array for high density immobilization of beads and tested the device with ϕ15 μm beads (Figure 1(b)). Our proposed design for hydrodynamic confinement is extremely efficient, thus highly suitable for handling small samples. Compared with previously reported hydrodynamic traps [2-4], fabrication is simple, and the design criterion allows one to design the device without any trial and error. With the same design criterion, we have also fabricated a high density (1×10^4) device for immobilization of beads. The actual trap-and-release device used in our experiments is shown in figure 4. This device is designed to immobilize 100 beads, and has traps that are numbered for individual addressability. To demonstrate the individual addressability of the bead microarray, and the ease of operation of our trap-and-release device, beads were arrayed and subsequently selected beads were released to form patterned lines. High speed camera images captured the instant at which a trapped bead was displaced by an optical-based microbubble (Figure 4). IR laser set to a power of 0.3 W was focused on the aluminum pattern (t=0.0 sec), and after 373 ms, bubble formation started. The expanding bubble displaced the previously immobilized bead into the main channel, where it was carried out of the array. This retrieval procedure typically took less than 0.6 s to complete. After the laser was switched off, the bubble cooled down, shrank and disappeared in about 3 s.

Cell-encapsulating Alginate Hydrogel microbeads

Figure 5(a) shows how the size of the cell encapsulating hydrogel beads varies with Q_c. Beads with length/diameter ranging from 100 -150 μm were obtained for the range of flow rates tested. All the beads were spherical (AR< 1.03, $C.V._{AR}$ < 3.0 %) with a narrow size distribution (C.V. < 3.2 %) apart for those produced at Q_c = 0.2 ml/h, which were discoidal (AR=1.2, $C.V._{AR}$ = 5.9 %) with a wider size distribution (C.V. = 5.7 %). We also studied how the concentration of $CaCO_3$ affected the viability of the cells (Figure 5(b)). Percentage of cells that remained alive after the encapsulation process increased from 19.3 % to 74.3 % when the $CaCO_3$ concentration was increased from 1.14 to 9.10 mg/ml solution. Here, trypan blue was used to differentiate live from dead cells. Figure 5(c) shows the cell encapsulating alginate hydrogel beads, and figure 5(d) shows the close-up of alginate beads containing live/dead Jurkat cells after trypan blue was added. Cells are selective in the compounds that pass through the membrane; trypan blue is not absorbed in a live cell, but it traverses the membrane in a dead cell. Hence, only dead cells will exhibit a distinctive blue color. Higher loading of $CaCO_3$ leads to higher crosslink densities, resulting in stiffer alginate hydrogels[20] that may help protect encapsulated cells from mechanical stresses during preparation. Increase in viability of the cells with the increase in $CaCO_3$ concentration is also attributed to the dual role played by $CaCO_3$. Besides releasing Ca^{2+} when the pH lowers, CO_3^{2-} is also released from $CaCO_3$. CO_3^{2-} acts as a base, regulating the pH inside the beads. Beads prepared with a higher concentration of $CaCO_3$ essentially have a larger reserve of CO_3^{2-} to prevent it from becoming overly acidic. Thus, we believe that both the increase in mechanical strength of the hydrogel and the milder internal environment subsequently translate to higher viability of the encapsulated cells.

Cell-based dynamic microarray

We formed a cell-based dynamic microarray by immobilizing cell-encapsulating alginate microbeads in a modified trap-and-release device (Figure 6). To demonstrate the release capability of the device, a cell-encapsulating alginate microbead was selected and released from the trap. Figure 6(a)-(c) shows the immobilized bead and its release process. The released bead was subsequently trapped in a trap farther downstream. The dotted line and arrow in Figure 6(d)

show the path taken by the bead after it was released from the trap. Trypan blue was then introduced into the device for 35 s before it was flushed out with RPMI buffer (Figure 6 (e)-(h)). During this process, the cell encapsulated in the bead that was immobilized in the adjacent trap (left) was stained rapidly by trypan blue, while cells in our bead of interest remained unstained, indicating that the release process did not compromise the viability of the encapsulated cells. With this experiment, we have successfully demonstrated that our dynamic microarray technology can be extended to handle cells, albeit cells were first encapsulated in alginate beads. Cells in culture are in different stages of the cell cycle and tend to exhibit high polydispersity in their size, direct trapping of the cells might result in multiple cells per trapping site. Encapsulation of cells in a biocompatible hydrogel matrix allows us to side-step all these problems and with the use of (RGD)-adhesive ligands incorporated alginates [5], we could further extend this dynamic microarray techology to handle adhesive cells in the near future.

6. CONCLUSIONS

Based on the criterion derived in this work, a dynamic microarray platform was designed, and fabricated using standard photolithography and soft lithography methods. Using microbeads, we first demonstrated the capabilities of our device. Such kinds of bead-based dynamic microarray hold great promise for advancing research in proteomics, diagnostics and drug discovery. In order to realize the cell-based arrays, we also successfully developed a cell encapsulation method in micro-devices that produced highly monodisperse alginate microbeads. By introducing mammalian cell encapsulating monodisperse alginate microbeads into a modified dynamic microarray device, we realized a platform for cell-based arrays. In our dynamic microarray device, although both approaches — hydrodynamic confinement and optical-based microbubbles — are presented in one device, they can also be separately utilized for other applications in microchip devices.

REFERENCES

1. D. Poncelet (2001) *Annals of the New York Academy of Sciences*, 944, 74-82
2. Yang, M. S., Li, C. W., & Yang, J. (2002) *Anal. Chem.* 74, 3991 -4001.
3. Wheeler, A. R., Throndset, W. R., Whelan, R. J., Leach, A. M., Zare, R. N., Liao, Y. H., Farrell, K., Manger, I. D., & Daridon, A. (2003) *Anal. Chem.* 75, 3581-3586.
4. Di Carlo, D., Aghdam, N., & Lee, L. P. (2006) *Anal. Chem. 78,* 4925-4930.
5. N. G. Genes, J. A. Rowley, D. J. Mooney, L. J. Bonassar (2004) *Arch. Biochem. Biophys.*, *442*, 161