HIGHLY EFFICIENT SINGLE CELL CAPTURING IN MICROWELL ARRAY USING HYDRODYNAMIC GUIDING STRUCTURES

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ABSTRACT

This paper presents a microfluidic chip array for high-throughput single cell assay with an efficient cell capture rate. The single cell capture structure utilizes hydrodynamic resistance difference in flow paths and its feasibility was tested. More than 40% of all the injected microbeads were captured in the designated capture sites. The capture structure was expanded in an array of microwells formed at the intersection of rows and columns of orthogonal microchannels. By selectively operating integrated pneumatic valves, we successfully demonstrated cell seeding, reagents injection and cell isolation without cross-contamination.

KEYWORDS: Single cell, High-throughput, Cell assay, Capture efficiency

INTRODUCTION

There have been growing interests in single-cell assay and a few groups have reported the microfluidic chips that incorporate single-cell capturing schemes [1-4]. However, their capturing efficiency (the ratio of the total captured cells to the injected cells) is relatively poor (less than a few %) and may not be adequate for handling rare cells such as stem cells or cancer cells. In this paper we propose a highly efficient hydrodynamic capture scheme which can be applied to microfluidic array chips for different reagent injection and cell isolation. Using this scheme, we can load single cells into each microwell and simultaneously apply different reagents for various single-cell assays.

WORKING PRINCIPLE

Figure 1 shows the overall schematic view of the proposed microfluidic chip. It consists of two PDMS layers: one for liquid fluidic channels and the other for control channels which open and close the fluidic channels using pneumatic membrane valves. We load cells vertically; inject reagents horizontally; and isolate cells in each microwell by addressing the integrated pneumatic valve array. For a higher capturing efficiency, we implemented a hydrodynamic guiding structure in each microwell as shown Figure 2. This structure will divide the flow path into two, path A and B, around a single cell capture site. Path A has a smaller flow resistance than path B; therefore, the injected cells are more likely to take the path A and the cells will be trapped in the capture site located at the center of the microwell. Once the cell is captured, path A will be blocked and accordingly its flow resistance becomes larger than path B. As a result, all the remaining cells will pass through the path B and be captured in the following microwells. This hydrodynamic capture structure can significantly increase cell capturing efficiency.

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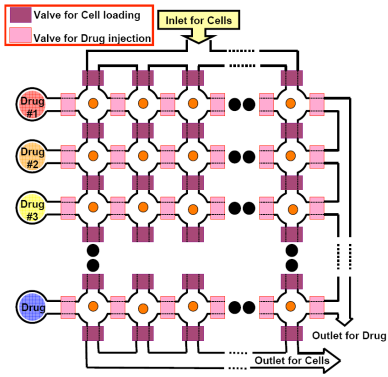

Figure 1. Schematic diagram of a microwell array.

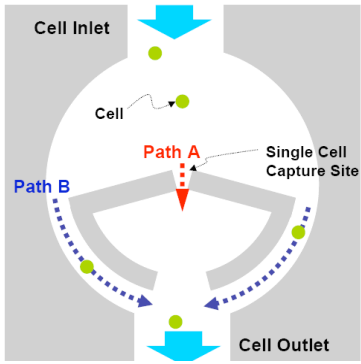

Figure 2. Schematic diagram of an effective single cell capturing structure.

EXPERIMENTAL RESULTS

Figure 3 shows the fabricated microchip. The microwell size is 400μm in diameter and flow channel is 150μm in width and 40μm in height. To verify the hydrodynamic capturing scheme, we performed the test by injecting microbeads (15μm in diameter, 1.8×10^5 beads/mL) at a flow rate of 1.5μL/min. It took less than 2 minutes for the microbeads to fill all the capturing sites. (Capturing movie clip can be found in [5].) Capturing efficiency over 40% was obtained by counting the captured beads with respect to all the injected beads. This is a significant improvement. Capturing efficiency without employing hydrodynamic capturing structures was observed less than 1~3% in our previous experiments [4]. We also captured living cells, myoblast stem cells, which are about 20μm in diameter. The captured cell image is shown in Figure 4. Different reagents (or drugs) can be simultaneously injected to the captured cells. Figure 5 shows the picture taken when four different color dyes are injected along every two rows, while each row was isolated from others by closing the horizontal valves at 15psi. We observed there was no cross-contamination for 48 hours. Figure 5B shows the photograph for microwell isolation test. This chip can be easily expanded to a larger array and will be adequate for massive-parallel loading of rare cells.

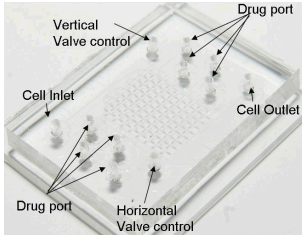

Figure 3. Photograph of the fabricated microfluidic assay chip.

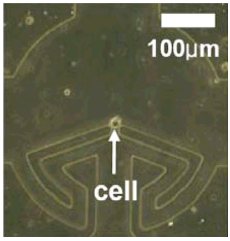

Figure 4. Photograph of the captured single myoblast stem cell.

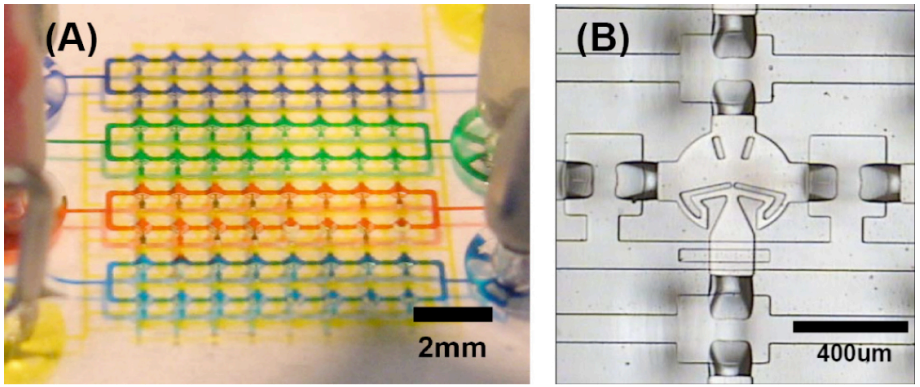

Figure 5. (A) Photograph of the drug injection test: Four different dyes are injected in every two rows and each row was isolated from others by closing the valves to prevent cross-contamination. (B) Photograph of microwell isolation test: All the valves are closed during isolation operation.

CONCLUSIONS

We proposed a hydrodynamic cell capturing scheme fabricated in a microfluidic array chip. Capturing efficiency has been significantly increased by simply deploying passive hydrodynamic guiding structures without using any complicated active schemes such as dielectrophoresis. We also demonstrated the massive-parallel loading of cells at very short period time, followed by drug injection and single cell isolation in each microwell. The proposed device can be easily expanded to a much larger array platform.

ACKNOWLEDGEMENTS

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