AN ELECTRONIC PIPETTE COMPATIBLE MICROFLUIDIC CHIP FOR CONTINUOUS PROCESSING OF SIZE-DEPENDENT CELL DEPLETION AND IMMUNOHISTOCHEMISTRY

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ABSTRACT

In this paper, a microfluidic chip for a continuous processing of size-dependent cell depletion by microfiltration and following immunohistochemistry for a specific antigen expressing on surfaces of separated cells is presented. The microfluidic chip can be connected directly with a conventional electronic pipettor for liquid handling and small enough (within 1 cm^3) to be dipped into a centrifuge tube for the processing. As a demonstration, size-dependent separation of U937 cells as target cell from a suspension containing red blood cells by using microfilter on the chip and following immunohistochemistry for CD45 on the cell surfaces of target cell is continuously conducted on the chip. The small size of the chip and the way of liquid handling using conventional electronic pipettor and centrifuge tube make the chip to be an easy-to-use tool for the processing without any other special equipment like centrifugal machine or syringe pump.

KEYWORDS

Cell separation, Cell size, Immunohistochemistry, Electronic pipette

INTRODUCTION

In the past two decades, microfluidic technology offers advanced tools for cell biology study [1, 2], reconstruction of tissues and organs *in vitro* [3], detection of rare tumor cells in blood [4] and so on. However, except for commercialized automated microfluidic systems [5], general use of microfluidic device for cell processing in common laboratory is still limited. One typical hurdle for this is difficulty in liquid handling on microfluidic devices using special and expensive equipment like syringe pump and complicated tubing. Here, we present a small microfluidic chip for continuous processing of size-dependent cell depletion by filtration and immunohistochemistry for a specific antigen on surfaces of separated cells by using a conventional electronic pipettor for liquid handling and centrifuge tubes for changing liquid used in the chip. This proposed chip allows the processing without any other special equipment like centrifugal machine or syringe pump and complex tubing for microvalves shown in conventional microchannel-based microfluidic devices.

EXPERIMENTAL

As shown in Figure 1A, the developed microfluidic chip can be connected to an electronic pipettor (Portable BD Falcon™ Express™ Pipet-Aid®, BD Biosciences) via a 2-mL pipette tip (Serological pipette, Nunc). The chip consists of a cap part to connect with the pipette tip and a microflilter layer made of PDMS, and a glass support (10-mm diameter) to seal microfilter structures on the microfliter layer (Figure 1B). The cap part equipped with a port of 3.5 mm works as connector between the pipette tip and the microfilter layer. In the microfilter layer, a reservoir port of 2 mm is fabricated to access the port on the cap part (Figure 1C). Figure 1D shows microfilter (25 μm width, 7.6 μm depth) on the chip. As shown in Figure 1D, a 10-μm (yellow) and a 16.5-μm (blue) fluorescent bead are successfully trapped by the microfilter.

Figure 2 shows schematic representation of continuous processing of size-dependent cell depletion and following immunohistochemistry using the chip. The depletion of small sized cells is conducted by pipetting (dispensing and aspiration) of sample cell suspension in a centrifuge tube containing appropriate buffer to remove smaller cells than the microfilter (Figure 2A). Following process of immunohistochemistry is implemented by aspirating a solution of fluorescent-labeled antibody, mixing and incubation (Figure 2B). The rinsing of unbound antibodies is conducted by the same manner with the previous depletion process (Figure 2C). Figure 1E shows actual image of the chip dipped in a 15-mL centrifuge tube (Centrifuge Tubes with Triple Seal Cap, Iwaki) for both cell depletion and rinsing process. To check the feasibility of the chip for the continuous processing of size-dependent cell depletion and following immunohistochemistry, a cell suspension containing U937 cells (Human leukemic monocyte lymphoma cell line; average cell size: 11 μm) as target cells and red blood cells (RBCs; average cell size: 6 μm) was used as a model cell suspension (1×10^6 cells/mL each, totally 0.9-mL). CD45 was chosen as a specific antigen expressing on the surface of target cells and a fluorescent-labeled anti-CD45 antibody (CD45 Mouse Anti-Human mAb (clone HI30), PE Conjugate, Life technologies) was used for the immunohistochemistry process. Centrifuge tubes containing of a culture medium of 5mL were used for both cell depletion and rinsing process of immunohistochemistry. In the cell depletion process and rinsing process of immunohistochemistry, pipetting (dispensing and aspiration) of cell suspension of 0.7 mL were repeated twice with the culture medium used as rinsing buffer. After the rinsing process of immunohistochemistry, cell suspension of 0.9 mL was enriched to 0.2 mL. Then the efficiency of the depletion of RBCs from the suspension and immunohistochemistry process was evaluated by using an image-base cytometer (Tali™, Life technologies).

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RESULTS AND DISCUSSION

To characterize flow in the chip generated by the electronic pipettor, DI water was used. The flow rates of dispensing and aspiration are calculated by the time required for processing 100-µl DI water. As shown in Figure 3A, the flow rates of dispensing and aspiration are 11.4 µl/s and 7.2 µl/s respectively. These flow rates are over one hundred smaller than that of the flow rates in pipette tip without the chip (data not shown). This accounts for the higher hydrodynamic resistance of the chip. Figure 3B shows histograms of cell size before and after the continuous processing of size-dependent cell depletion and following immunohistochemistry of CD45 of separated cells. The black bars in Figure 3B show the cell number of RBCs of 6 µm and U937 cells of 11 µm. Figure 3C shows relative cell number of RBCs of 6 µm against U937 cells of 11 µm. The concentration of both RBCs and U937 cells in sample suspension is initially same concentration at 1×10^6 cells/mL, however, the relative number of RBCs at before the processing is larger than that of U937 cells. This is because higher uniformity of cell size of RBCs than that of U937 cells. As shown in Figure 3C, the relative number of RBCs is decreased by the cell depletion process on the chip (before: 1.30 and after: 0.76). After the depletion process, CD45 (leucocyte common antigen) expressed on the separated cells are stained with PE-conjugated anti CD45 antibodies (incubated for 10 min at room temperature). Shift of fluorescence signal by the immunohistochemistry process are successfully observed as shown in Figure 3D (cytometry) and Figure 3E (fluorescence images). The continuous processing were performed within 30 min including 10-min incubation of immunohistochemistry.

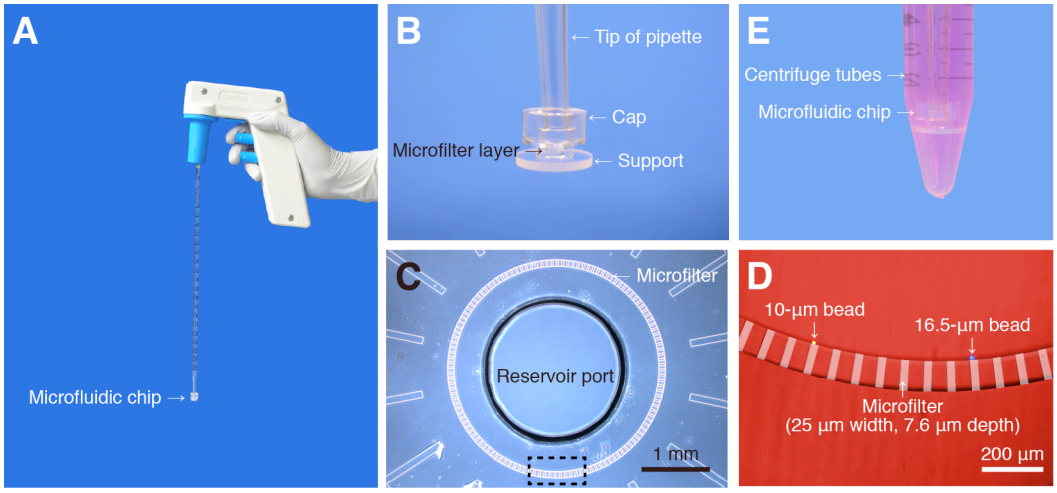

Figure 1. Microfluidic chip for size-dependent cell depletion and immunohistochemistry. (A) Photograph of the microfluidic chip connected to the electronic pipettor via the 2-mL pipette. (B) Magnified photograph of the chip. The chip consists of the cap and the microfilter layer made of PDMS, and the glass support. The access port of 3.5-mm diameter on the cap is connected with the tip of the pipette. (C) Micrograph of the microfilter layer. 2-mm reservoir port is punched on the microfilter layer. (D) Magnified photograph of the microfliter structures (white colored area) shown in dotted line square in Figure 1C. The width and depth of the microfilter are 25 µm and 7.6 µm respectively. A 10-µm (yellow) and a 16.5-µm (blue) fluorescent bead are trapped by the microfilter. (E) Microfluidic chip dipped in the centrifuge tube. Exchange of liquid inside the chip is implemented by dipping the chip into the centrifuge tube containing desired solution and following pipetting.

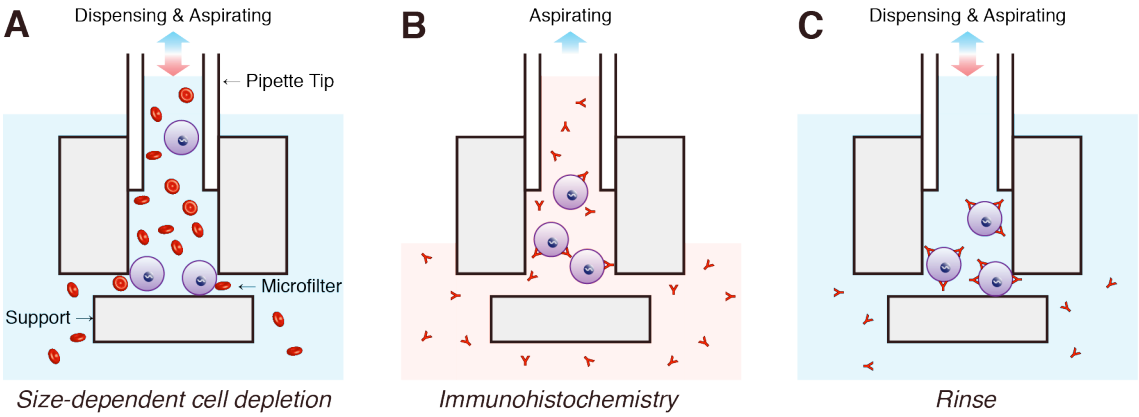

Figure 2. Schematic representation of the continuous processing of (A) size-dependent cell depletion, (B) immunohistochemistry and (C) rinsing to remove unbound antibodies. All liquid handling is implemented by combination of both manipulation of electronic pipettor and changing of centrifuge tubes.

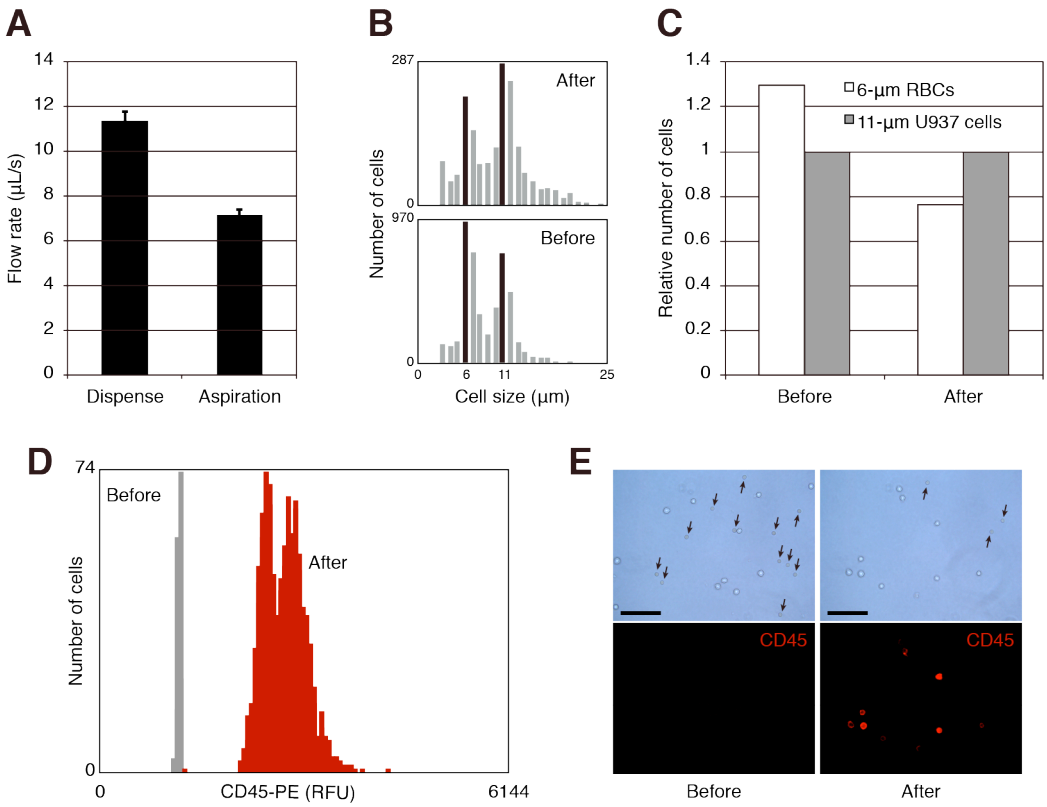

Figure 3. Results of flow characterization of the chip and continuous processing of size-dependent cell depletion and following immunohistochemistry of CD45. (A) Flow rates of DI water in the chip generated by the electronic pipette. The speed range of the pipettor is set to "Fast" mode. The error bars represent standard deviations from these measurements (N = 3). (B) Histograms of cell size before and after the continuous processing. Black colored bars in the histograms show average cell size of RBCs (6 μm) and U937 cells (11 μm) respectively. (C) Relative number of RBCs against U937 cells before and after the processing. The number of RBCs of 6 μm is normalized by the number of U937 cells of 11 μm. (D) Cytometry data before and after the processing. For the immunohistochemistry, PE-conjugated anti-CD45 antibodies were used. (E) Transmission (upper) and fluorescent (lower) image pairs before and after the processing. The arrows in the transmission images show RBCs. (Scale bars: 100 μm)

CONCLUSION

In this paper, we report a microfluidic chip for a continuous processing of size-dependent cell depletion by microfiltration and following immunohistochemistry for a specific antigen expressing on separated cells without using expensive and special equipment like centrifuge machine and syringe pump. Since all liquid handling carried out by using just electronic pipettor and centrifuge tubes that conventionally and generally used in usual laboratory, we believe the compatibility of the chip for experimenter is higher than that of microfluidic devices developed previously. Although improvement of purity for target cell after cell depletion process should be required, with the improvement, for example, the developed microfluidic chip would be further applied to a rapid and easy tool to isolate of leukocytes from peripheral blood.

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