A SIMPLE METHOD FOR CELL ISOLATION BY UTILIZING BOTH CELL SIZE AND AFFINITY TO SURFACES

Ayako Araki[1, 2], Shohei Kaneda[1, 2], Teruo Fujii[1, 2]

[1]Insitute of Industrial Science, The University of Tokyo, Japan, [2]JST-CREST, Japan

ABSTRACT

In this paper, we propose a novel method to separate cells larger than arbitrary cell size or cells expressing a specific antigen by combining both size-based cell separation using microfilter and immunoaffinity-based cell separation using an antibody-coated surface. The proposed method consists of two processes: (1) a filtration process to capture target cells having larger cell size than a microfilter or cells expressing a specific antigen and (2) a rinsing process to remove non-target cells having smaller cell size. The effectiveness of the method was validated through experiments of cell separation on a microfluidic chip using a cell suspension of MCF-7 cells (as model cells with a specific antigen) and U937 cells (without a specific antigen). In addition, the relationship between number and position of captured cells during the cell separation process is discussed.

KEYWORDS

Cell isolation, Cell size, Immunoaffinity, Cell separation

INTRODUCTION

Although microfluidic approaches have been offering various size-based cell separation methods [1] and immunoaffinity-based cell separation methods [2], there are a few reports about the method combining those two ways [3]. For example, in the case of isolation of CTCs (Circulating Tumor Cells) from peripheral blood, it is pointed out that variability of expression of antigens on surfaces of CTCs and variability of cell size of CTCs sometimes cause low capture efficiency [3]. Therefore, we propose a novel method for cell separation based on both cell size and immunoaffinity.

CONCEPT

In this proposed method, both a microfilter and an antibody-coated surface are used for size-based and immunoaffinity-based cell separation respectively. Here cells larger than a microfilter or cells expressing a specific antigen are target cells. Firstly, a heterogeneous cell suspension is applied to the microfilter and the antibody-coated surface of the substrate as shown in Figure 1a. By aspirating the cell suspension via the microfilter, cells with a specific antigen are captured onto the surface and larger cells without the antigen are captured at the edge of the microfilter, whereas smaller cells without the antigen go through the microfilter (Figure 1b). Thus both size-based and immmunoaffinity-based cell separation are conducted as filtration process. Then to collect the remaining smaller cells without the antigen, an additional buffer solution is applied to the surface and aspirated as shown in Figure 1cd (rinsing process). Finally the target cells having larger cell size or the immunoaffinity to the surface are separated (Figure 1e).

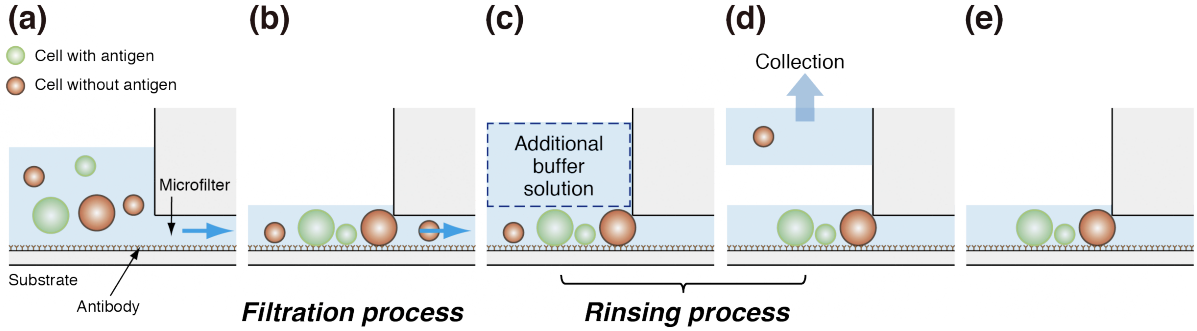

Figure 1. Conceptual schematics of separation of target cells by combining size-based and immunoaffinity-based cell separation. Antibodies for a specific antigen are immobilized on the surface of the substrate. (a) Cells are transported to the surface and the edge of the microfilter. (b) Filtration process. Both size-based and immunoaffinity-based cell separation are realized. Cells larger than the microfilter are captured at the edge of the microfilter. Cells with the specific antigen are captured onto the surface. Smaller cells without the antigen go through microfilters. (c, d) Rinsing process. To collect the remaining smaller cells without the antigen, the additional buffer solution is applied (c). Then the solution is aspirated to collect the remaining cells (d). (e) Cells with the antigen and cells larger than the microfilter are separated.

EXPERIMENTAL

To check the feasibility of the proposed method, a microfluidic chip shown in Figure 2 was fabricated. The chip is composed of a reservoir and microfilters made of PDMS, and a glass substrate (Figure 2a). In microfilters, 50-μm square-shaped microstructures with 13.4-μm depth are arrayed for size-based cell separation (Figure 2bc). The diameter of the reservoir is 3 mm and anti-EpCAM antibodies are immobilized onto the surface of the substrate.

978-0-9798064-5-2/μTAS 2012/$20©12CBMS-0001

As a demonstration, experiments of cell separation using a cell suspension containing MCF-7 cells (cell size: 16 μm, with EpCAM expression) and U937 cells (cell size: 13 μm, without EpCAM expression) were conducted on the chip. A chip without antibody-coated surface (PDMS surface) was used as control. The experimental set up of the chip is illustrated in Figure 2d. A laboratory-made container is used to hold the chip (Figure 2d). A PTFE tube inserted into the bottom of the container is connected to a syringe pump to aspirate cell suspension in the reservoir thorough microfilters (Figure 2d). A tube placed above the chip is used for rinsing process. Culture medium supplied from an electronic pipette via the tube is used as an additional buffer solution at rinsing process. In the experiments, firstly the cell suspension of 10 μl was introduced into the reservoir. Then culture medium of 600 μl was applied into the container to avoid the exhaustion of liquid in the reservoir during filtration process. Then static incubation for 3 min was conducted. As filtration process, culture medium of 300 μl was aspirated and dispensed through the tube connected to the electronic pipette and subsequently aspiration of the cell suspension in the reservoir at flow rate of 30 μl/min for 1 min was conducted. Then rinsing process was implemented by pipetting operation (ten-times of aspiration and dispensing of the additional buffer solution of 300 μl for 24 sec) and aspiration of the buffer solution of 400 μl to collect the remaining cells without immunoaffinity to the surface. Fluorescent images of the chips were obtained during the filtration and rinsing process to count cells on the surfaces of the substrate.

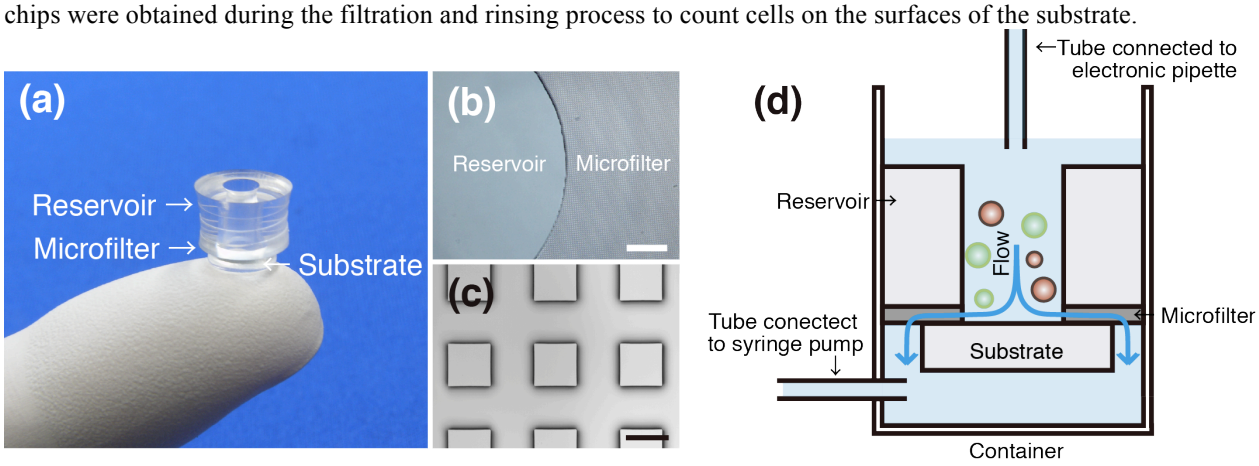

Figure 2. Fabricated microfluidic chip. (a) The chip consists of a reservoir and microfilters made of PDMS, and a glass substrate. (b) Micrograph of the microfilter part. The scale bar is 500 μm. (c) Magnified image of microstructures working as microfilters. The scale bar is 50 μm. (d) Schematic drawing of experimental set up. The microfluidic chip is held in the container. The upper tube is connected to an electronic pipette for rinsing process. The lower tube is connected to a syringe pump for filtration process.

RESULTS AND DISCUSSION

Figure 3 and Table 1 show results of the experiments of cell separation. The dotted line circles in Figure 3 show the edge of the microfilters on the chips. Green and red fluorescence represent MCF-7 cells and U937 cells respectively. Figure 3a shows images of before and after filtration process and following rinsing process on the antibody-coated surface. Figure 3b shows images of the PDMS surface. After the incubation for 3 min, most MCF-7 cells were captured on the antibody-coated surface, whereas U937 cells were moved toward the edge of the microfilters during the filtration process (Figure 3a middle) and depleted by the rinsing process (Figure 3a right). White colored arrows shown in Figure 3a are indicated U937 cells keeping the same positions at the edge of the microfilters during the filtration and rinsing process. Note that with the present condition of the rinsing process, U937 cells captured at the edge of the microfilters were not collected by the rinsing process (indicated by the arrows in Figure 3a). We assumed there are two possibilities: (1) embedding of the captured U937 cells into the microfilter may be induced by the flow in the filtration process (flow rate: 30 μl/min) and (2) the low efficiency of rinsing at the edge of the microfilters. During the cell separation process, cells on the surfaces are counted and recovery rates of both MCF-7 cells and U937 cells were calculated as shown in Figure 4a. Recovery rates of MCF-7 cells on the antibody-coated surface were kept over 93 % during the processes (after filtration process: 99%, after rinsing process: 93%), whereas recovery rates of MCF-7 cells on the PDMS surface were less than 80%. From the decreasing of recovery rate of U937 cells on the antibody-coated surface, the effectiveness of the rinsing process was confirmed. Figure 4b shows the percentage of cells on the surfaces. The percentage of MCF-7 cells on the antibody-coated surface was increased during the filtration and rinsing processes, whereas the percentage of MCF-7 cells on the PDMS surface was almost kept constant. This is because that the U937 cells located at except for the edge of the microfilters on the antibody-coated surface are almost collected by the rinsing process.

CONCLUSION

We proposed a simple cell separation method combining size-base and immunoaffinity-based cell separation. By using the proposed method, cells larger than microfilters or cells expressing a specific antigen are successfully separated on a microfluidic chip. We believe this method can be applicable to various cell separations such as isolation of CTCs or CD4+ T cells from peripheral blood.

Table 1. Results of the number of MCF-7 cells and U937 cells on the surfaces during the cell separation processes.

	Before filtration		After filtration		After rinsing	
	MCF-7	U937	MCF-7	U937	MCF-7	U937
Antibody-coated surface	107	54	106	31	100	9
PDMS surface	66	51	50	13	47	11

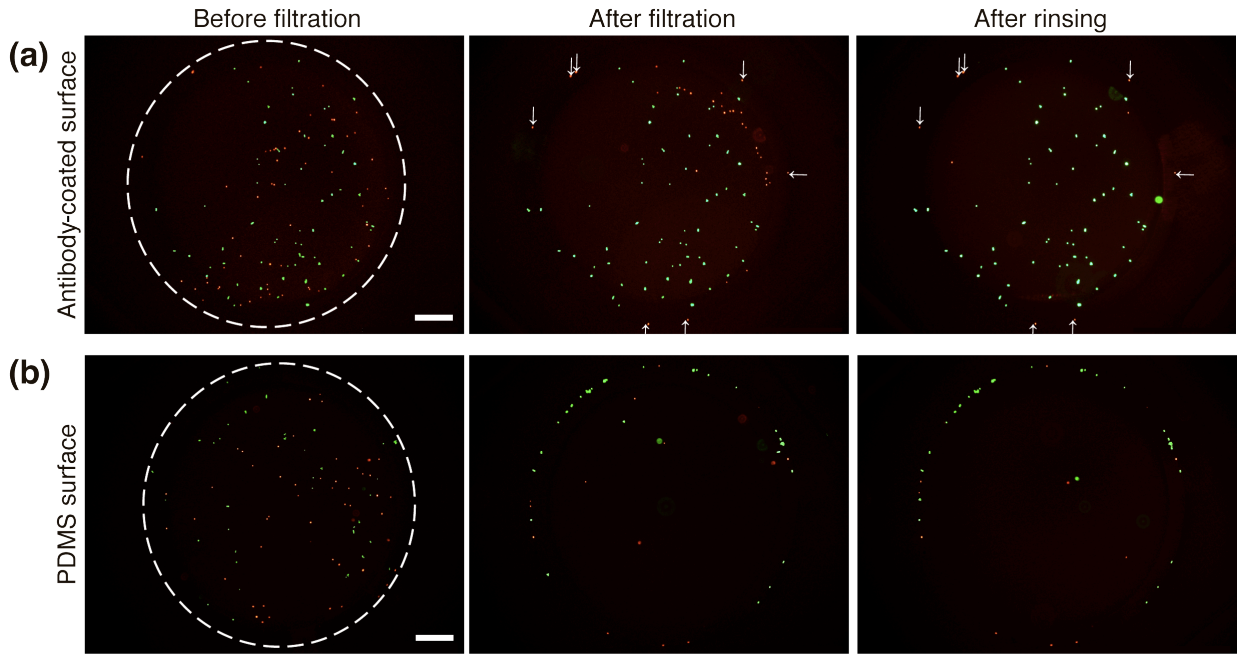

Figure 3. Results of cell separation using a cell suspension containing MCF-7 cells (green) and U937 cells (red) performed on the chip with (a) the antibody-coated surface and (b) the PDMS surface (without antibody). During the cell separation process, fluorescent images are obtained at before filtration, after filtration and after rinsing process. White colored dotted line shows the edge of the microfilters. White colored arrows in Figure 3a indicate U937 cells keeping the same positions during the filtration and rinsing process. The scale bar is 50 μm.

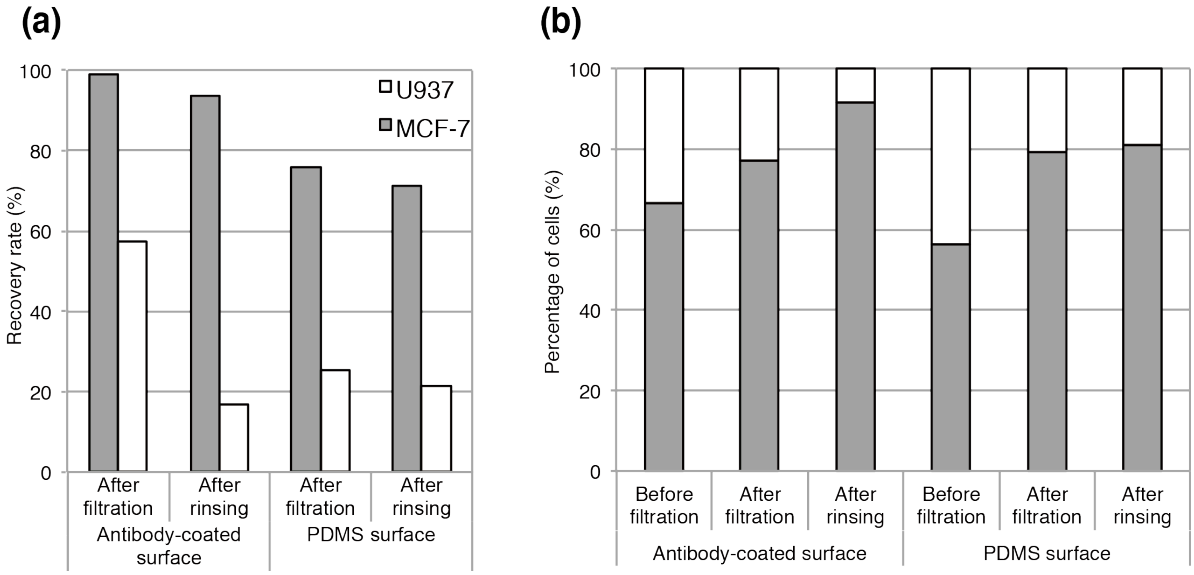

Figure 4. Results of recovery rates and percentage of MCf-7 cells and U937 cells during the cell separation process. (a) Recovery rates. (b) Percentage of cells on the surfaces.

REFERENCES

[1] S. Zheng *et al.*, *Biomed. Microdevices*, pp. 203-213, 13 (2011).
[2] S. L. Stott *et al.*, *PNAS*, pp. 18392-18397, 107 (2010).
[3] P. J. Maimonis *et al.*, *Proc. AACR* (2010).

CONTACT

Teruo Fujii tfujii@iis.u-tokyo.ac.jp