MICROFLUIDIC CELL SEPARATION WITH ANTIBODY MODIFIED EUGLENA BY USING PHOTOTAXIS MEDIATED MIGRATION
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ABSTRACT
We develop a microfluidic cell separation method which combined microfluidic devices and multifunctional Euglena. Multifunctional Euglena for cell separation was successfully prepared by the surface modification of Euglena cell membrane and its performances were confirmed. Mutlifunctional Euglena in the inlet attached cells, migrated inside the microchannel by phototaxis towards the outlet chamber, and brought specific cells to the outlet. After moving to the outlet, cells in the outlet were detached from Euglena. Since it is based on the unique characters of Euglena, our method needs only simple device and can deal with small to large amount variety kinds of samples including cells.

KEYWORDS: Microdevice, Euglena, cell separation, surface modification

INTRODUCTION
A cell separation technique is significantly important in the clinical diagnosis and the regenerative medicine. Fluorescence activated cell sorter (FACS) and magnetic beads activated cell sorter (MACS) have been employed in the cell separation as well as the centrifugation method. In deed, FACS and MACS play significant role in especially separation of specific cells. However, these methods require high cost and skilled technicians. In addition, MACS can not deal with a large amount of cells. Therefore, development of a new cell separation method still has been demanded, which enables ease, low cost, highly specific and from large to minute amount cell handling. In this paper, we focused on the properties of Euglena, which shows phototaxis, and applied it to cell separation in order to develop a new cell separation method.

PRINCIPLE
The principle of cell separation with Euglena is shown in Figure 1. For separation of target cells, we prepared surface modified Euglena, which can carry only the target cells, and utilized Euglena’s phototaxis. This surface modified Euglena forms Euglena-target cell complex when Euglena is added into the sample. After this sample is injected into the inlet of straight microchip and is exposed to light, Euglena-target cell complex starts to migrate toward the opposite side of inlet and cell separation could be attained.

EXPERIMENTAL
Poly(dimethylsiloxane) (PDMS) straight microchannels of different size were prepared by the soft lithography method. The microdevice for cell separation as shown in Figure 2 was prepared by bonding glass plate and PDMS straight channel. The microdevice surface was treated with 1.0wt% bovine serum albumin solution to reduce adsorption of Euglena and cells onto the microdevice surface. The surface modification of Euglena was performed according to Iwata et al and Nagamune et al’s paper [1, 2]. The surface modification was confirmed by the confocal laser fluorescence microscopy and spectrofluorometer.

Figure 1. Principles of cell separations with Euglena

Figure 2. Picture of the microdevice for cell separation
Microchannel height, length and width are 50 μm, 5 cm and 50 μm, respectively.
RESULTS AND DISCUSSION

Apparent velocities of *Euglena* were measured with different width microchannels, different wavelength of the light, and different light intensity. Figure 3 indicates that the apparent velocity depended on the microchannel width. Similarly, apparent velocity also depended on the wavelength of light and light intensity as shown in Figure 4 and Figure 5. These dependencies are thought that *Euglena* was made go straight in the narrower channel and has phototaxis depending on the wavelength of light and light intensity.

The surface modification of *Euglena* was attempted with fluorescein-5-isothiocyanate (FITC) conjugated molecules and was confirmed with the confocal laser fluorescence microscopy and the spectrofluorometer. The confocal laser fluorescence microscopy image in Figure 6 shows that *Euglena* surface was successfully modified with FITC. In addition, stability of the surface modification was accessed with the spectrofluorometer. Figure 7 indicates that stable surface modification of *Euglena* was successfully attained with these methods though a small amount of immobilized FITC conjugated molecules was gradually detached from the *Euglena* surface. Furthermore, the viability tests of *Euglena* after surface modification were performed and revealed that these surface modification methods have no significant damage on the viability of *Euglena*. Therefore, these surface modification methods permit us to prepare multifunctional *Euglena* with ease and short time.

Finally, we attempted to separate stimulated cell, which is biotin modified fluorescence polymer particles, with streptavidin modified *Euglena*. The results indicate that separations of stimulated cells and purification of them up to 90% were attained. In addition, after the separation, stimulated cells binding *Euglena* were detached from *Euglena*. Thus, with the surface modified *Euglena*, we can easily separate and obtain target cells from other cells. In addition, our method could deal with small to large amount of variety kinds of sample because our method requires only surface modified *Euglena* and light irradiations.

CONCLUSION

We successfully developed a new separation method with multifunctional *Euglena* and the microdevice. We revealed that culture conditions (not described in this manuscript), microchannel width, the wavelength of light and light intensity largely affect the separation efficiency in our developed method. Our developed method requires only multifunctional *Euglena*, light irradiation and the microdevice. Therefore, our developed method permits us to separate cells with ease, low cost and high specificity and would be useful in bed side.
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