MICROFLUIDIC RARE CANCER CELL COLLECTION WITH ANTI-EpCAM ANTIBODY MODIFIED *EUGLENA* BY PHOTOTAXIS INSIDE MICROCHANNELS

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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 skillful 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 depending on the purpose. Since we have reported cell separation with multifunctional *Euglena* [1], we have been attempting real cell separation with our developed method. In this paper, we introduce our recent results, especially cell separation in blood cells.

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.

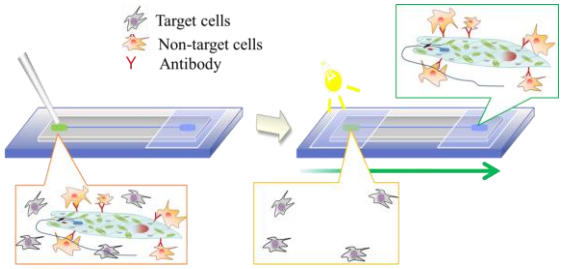

Figure 1. Principles of cell separations with Euglena

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 [2, 3]. The surface modification was confirmed by the confocal laser fluorescence microscopy and spectrofluorometer.

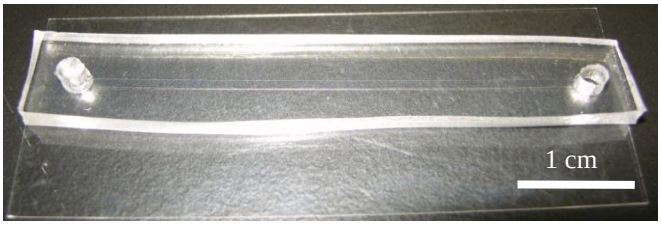

Figure 2. Picture of the microdevice for cell separation Microchannel height, length and width are 50 μm, 5 cm and 50 μm, respectively.

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 phtotaxis 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 stereptavidin 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. Furthermore, we attempted to separate real cells in blood. Figure 8 shows that surface modified *Euglena* can carry the cells in the blood. Though this is preliminary results, 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.

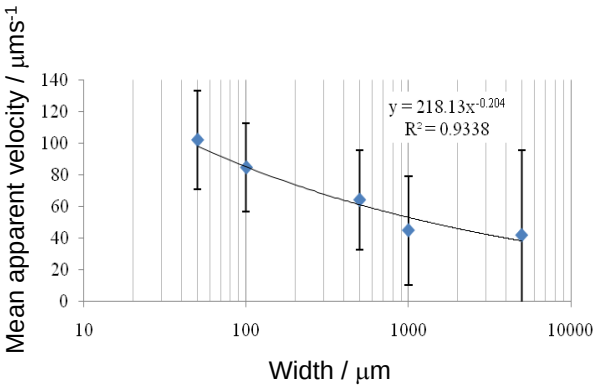

Figure 3. the dependency of the apparent velocity of Euglena on the microchannel width

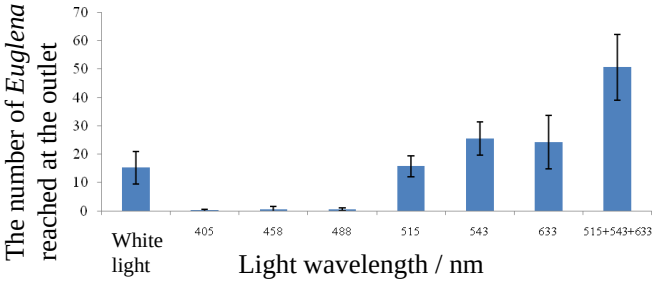

Figure 4. the dependency of the apparent velocity of Euglena on the irradiated light wavelength

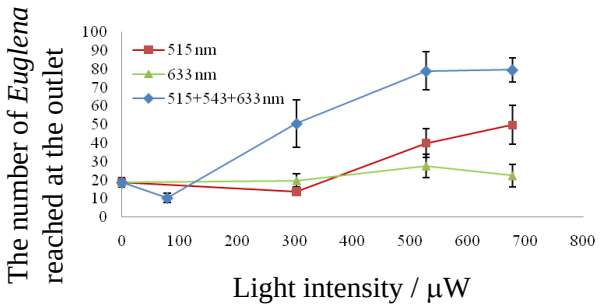

Figure 5. the dependency of the apparent velocity of Euglena on the light intensity

CONCLUSION

We successfully developed a new separation method with multifunctional *Euglena* and the microdeivce. 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.

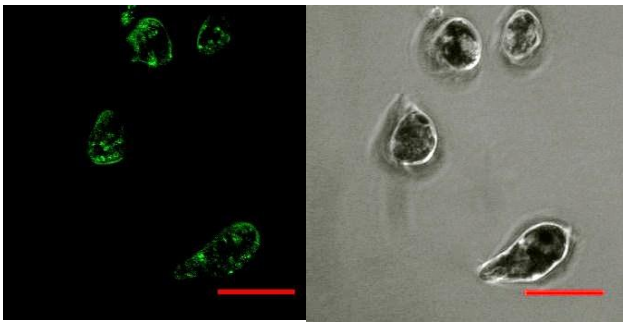

Figure 6. Confocal microscopy and microscopy images of Euglena modified with FITC molecules. Scale bar: 20 μm

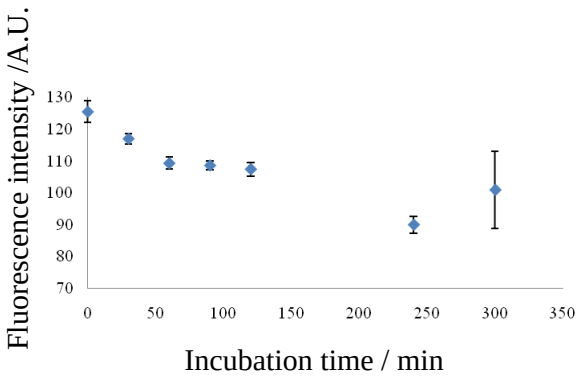

Figure 7. Stability of surface modification

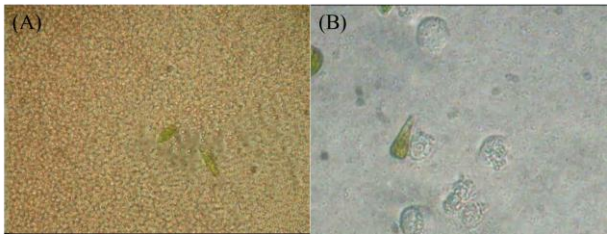

Figure 8. Microscope images of (A) swimming Euglena in blood and (B) the complex between antibody immobilized Euglena and lung cancer cell

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REFERENCES

[1] Y.Okamoto *et al*. *Micro Total Analysis Systems 2011*, 1909-1911(2011).
[2] S. Miura, Y. Teramura, H. Iwata, *Biomaterials*, **27**, 5828–5835(2006)
[3] K. Kato, C. Itoh, T. Yasukouchi, T. Nagamune, *Biotechnol. Prog.* **20**, 897-904(2004).

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