ADHESION-BASED CELL VELOCITY REGULATION IN AN ANTIBODY-COATED MICRO COLUMN FOR STEM CELL SEPARATION

Junichi Miwa, Yuji Suzuki and Nobuhide Kasagi

Department of Mechanical Engineering, The University of Tokyo, Japan

ABSTRACT

We report the development of a prototype adhesion-based cell separation device for precise extraction of stem cells from a small amount of concentrated sample, a key issue in regenerative medicine. The cell separation method mimics the mechanism of leukocyte recruitment from blood vessels at sites of inflammation, and the device consists of an antibody-coated micro column for regulation of cell velocity by antigen/antibody interaction. Antibody immobilization on the micro channel walls is accomplished by conjugation of biotin on aminomethyl-conjugated parylene surface. Results of cell velocity measurement in antibody-coated channels show that the target cells are slowed down to approximately half the flow velocity, showing the effectiveness of the cell separation principle.

Keywords: stem cells, cell sorting, functionalized parylene, antigen/antibody interaction

1. INTRODUCTION

Tissue engineering is a promising biomedical technology in the near future, enabling us to replace damaged parts of the body with cells or tissue that are cultivated *in vitro*. Multipotent stem cells are a strong candidate for the source of regenerated tissue, and considering ethical issues, use of somatic stem cells is preferable to the use of embryonic stem cells. Mesenchymal stem cell (MSC), derived from adult bone marrow or blood, has the ability to proliferate indefinitely or differentiate into various kinds of cells [1]. However, MSC is very rare, with a number density of 10 cells/m*l* in peripheral blood, giving rise to the need for a safe, inexpensive, and highly efficient method for separating stem cells from whole blood or bone marrow.

Currently the most efficient way to separate a rare cell from a suspension of multiple cell types is to recognize its specific surface antigen. Microfluidic devices capable of cell separation based on surface antigens have already been developed [2, 3], but they require electric or magnetic fields which may affect the unspecialized characteristics of the stem cells. In this paper, we report the development of a prototype adhesion-based cell separation device with a separation principle requiring only antigen/antibody reaction. The working principle consists of only a single step, and holds the potential to perform cell separation with more simple structure of the device.

2. CELL SEPARATION PRINCIPLE

Figure 1 shows the schematic of the present cell separation method. The working principle mimics the mechanism of leukocyte recruitment from blood vessels at sites of inflammation, where the leukocytes slow down by transient attachment and detachment of antigen/antibody bonds between the cell membrane and the endothelium. By coating the antibody on the channel walls with a sufficient surface density, it is expected that the

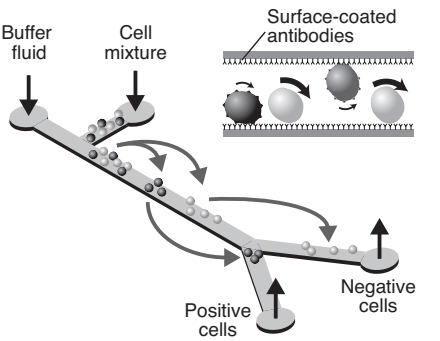

Figure 1. Schematic of the adhesion-based cell separation method.

0-9743611-1-9/□TAS2005/$20©2005TRF

target cells slow down to a terminal velocity, at which they could be completely separated from the non-target cells. Recently, leukocyte trapping in a micro channel with antibody-coated pillars has been reported [4], but a problem lies in extracting the trapped cells from the micro device. The present work aims at separating the cells without trapping, in order to eliminate the need of releasing agents such as trypsin for extraction of cells from the device.

3. FABRICATION AND EXPERIMENTAL

Functionalized parylene [5] is a strong candidate for the surface on which biomolecules are immobilized. In the present study, diX AM (poly(aminomethyl-[2,2]paracyclophane), KISCO), a new class of functionalized parylene with aminomethyl group (Fig. 2a), is selected as the material for the antibody-coated micro channel walls. The advantage of using functionalized parylene for biomolecule immobilization is two-folds. Firstly, parylene is biocompatible in nature, and secondly, amino group is available on the conformally-coated surface straight after vapor deposition. The micro channel structure is made by standard lithography processes of SU-8, followed by vapor deposition of parylene C and diX AM on the entire surface of the structure. The channel is closed by bonding the channel structure and a pyrex wafer, also coated with parylene C and diX AM, at 200 °C under vacuum [6]. Figure 3 shows the microfabricated prototype cell separation micro column. For all the target cells to be affected by antigen/antibody interaction, the channel should be designed so that all the cells flow in the vicinity of the wall. To meet this requirement, the channel height is made 50 μm, which is approximately twice the diameter of typical rare cells found in blood. The channel width was made sufficiently large to avoid clogging of cells in the channel, i.e., 100 μm in the present study. The desired column length varies depending on the surface density of antibodies on the wall. Here, the column length is varied from 40 to 200 mm.

The antibody immobilization method is shown in Fig. 2b. NHS-LC-LC-biotin is conjugated to the surface amino group of diX AM through 1 hour incubation at 30 °C, and biotin-conjugated antibody is immobilized on the surface, mediated by streptavidin. A combination of human umbilical cord vein endothelial cell (HUVEC) and mouse anti-human biotin-conjugated CD31 (PECAM1) monoclonal antibody is chosen as the target cell and its counterpart antibody, for verification of the current cell separation principle. Human mesenchymal stem cell

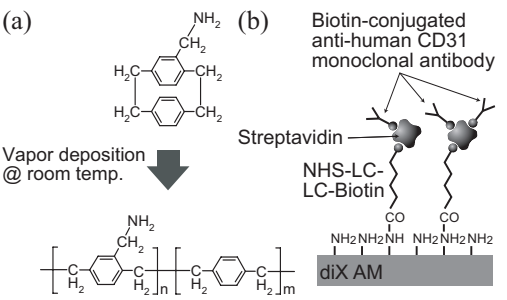

Figure 2. (a) Polymerization of diX AM, (b) Antibody immobilization on the micro channel walls.

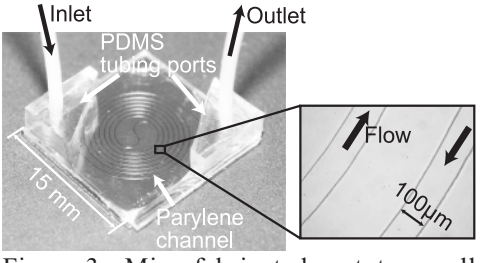

Figure 3. Microfabricated prototype cell separation column.

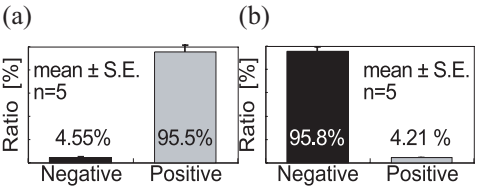

Figure 4. CD31 expression of (a) HUVEC and (b) hMSC, investigated by autoMACS (Miltenyi Biotec GmbH) [3].

(hMSC) is used to represent CD31-negative cells. Figure 4 shows CD31 expression of each cell type [3]. Cell suspensions are introduced into the micro column at a number density of 10^6 cells/m*l*, and cell velocities are measured from microscopic images at flow rates varied from 0.01 to 0.5 μ*l*/min, corresponding to the bulk mean velocity of 0.04 mm/s to 2 mm/s.

4. RESULTS AND DISCUSSION

Figure 5 shows microscopic images of cell motion at the volumetric flow rate of 0.01 μ*l*/min. There is apparent difference between the velocities of the two cells, with the velocity of HUVEC estimated from microscopic images being approximately half of that of hMSC. This result confirms the working principle of the current cell separation column, showing that a cell sample containing multiple cell types flows downstream to form separate plugs with target and non-target cells. To confirm that the reduction in HUVEC velocity is due to the presence of antibodies at the micro channel walls, velocity measurements from microscopic images are made in micro columns with and without antibody coating (Fig. 6). The velocity of HUVEC in the antibody-coated column is 1/5 to 1/2 of that in a plain column, confirming the effectiveness of the present cell separation principle. The effect of antigen/antibody interaction holds at any flow rate investigated, and this fact leaves the possibility of cell separation at higher flow rates.

5. CONCLUSION

The concept of adhesion-based cell separation by transient antigen/antibody interaction is proposed, and a prototype micro cell separation column with functionalized parylene-coated micro channel walls is fabricated. Results from cell velocity measurements show that target cells flow downstream at approximately half the velocity of the surrounding flow, confirming the effectiveness of the present cell separation principle.

ACKNOWLEDGEMENTS

We would like to express gratitude to Professors T. Ushida and K. Furukawa for cultivation and preparation of cells. This work was supported through Grant-in-Aid for Scientific Research (S) (No. 15106004) by MEXT, Japan. J.M. also acknowledges the financial support from the 21st Century COE Program, "Mechanical Systems Innovation" by MEXT.

REFERENCES

[1] Y. Jiang et al., Nature, 418, pp. 41-49, (2002). [2] A. Y. Fu et al., Nature Biotech., 17, pp. 1109-1111, (1999). [3] W. H. Tan et al., μTAS '04, 2, pp. 156-158, (2004). [4] L. P. Lee and D. Liepmann, Lab on a Chip, 5, pp. 64-73 (2005). [5] J. Lahann et al., μTAS '02, 1, pp. 443-445, (2002). [6] H. S. Noh et al., J. Microelectromech. Sys., 11, pp. 718-725 (2002).

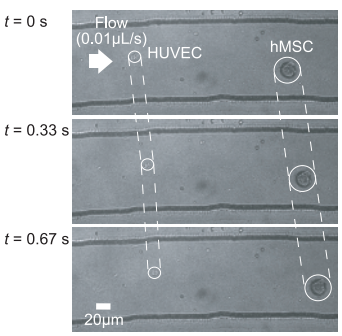

Figure 5. Microscopic images of HUVEC and hMSC in the antibody-coated micro column.

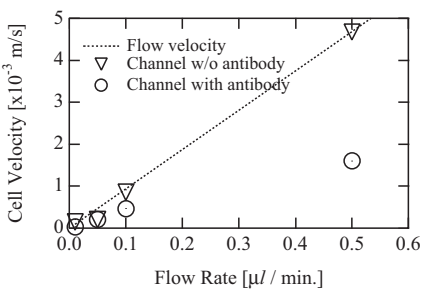

Figure 6. Comparison of the velocities of HUVEC in micro channels with and without surface-coated antibodies.