ABSTRACT

Blood analysis plays an important role in health care, although conventional technology is time-consuming. Previously, our group have developed an automatic microfluidic ELISA device (µELISA) for rapid analysis with low cost. In addition, we have developed microfluidic plasma separation technique and combined to µELISA to realize whole blood immunoassay. In this paper, we applied the whole blood immunoassay to a real sample (C-reactive protein, CRP) and verified its availability for clinical use.

KEYWORDS: Plasma separation, whole blood, point of care testing

INTRODUCTION

Blood analysis is essential for clinical diagnosis, health monitoring and disease research due to its numerous components such as proteins, fats and sugars. However, conventional blood analysis requires ~10 ml sample and long time for plasma separation by centrifuging and analysis using big instruments. Therefore, we have developed µELISA to decrease the sample volume (~µL) and analysis time (~min) [1]. Nevertheless, plasma separation for the minute volume has not been established though many methods such as Zweifach-Fung effect or membrane filtering have been reported [2]. Previously, we have developed a plasma separation device combining the microfluidic effect and filtering firstly and succeeded in proof of concept [3]. In this study, the validity of the device for CRP in whole blood was examined using µELISA.

THEORY

The principle of the plasma separation device is shown in figure 1. Blood cells in a upper microchannel receive lifting force by Safman force and concentrated in the center of the microchannel. This effect is called axial migration. Then, a plasma layer is formed near the microchannel wall and filtered by a porous membrane. Because the flow directions of whole blood and plasma are perpendicular, this configuration can prevent clogging of blood cells and hemolysis significantly.

Figure 1: Principle of plasma separation.
**EXPERIMENTAL**

The device was assembled as shown in figure 2. A polycarbonate membrane was sandwiched by two glass substrates with microchannels (300 μm wide and 140 μm depth) and aluminum holders. The surface of the glass substrates were modified with hydrophobic molecules (octadecyltrichlorosilane) to generate Laplace pressure in the gap of substrates and the membrane, which increased the withstanding pressure of the device. The length of the blood channel was designed to be 10 mm to give enough time for axial migration of red blood cells from the result of a finite element calculation.

![Figure 2: Plasma separation device.](image)

**(a)** Polycarbonate porous membrane was sandwiched by two glass substrates and aluminum holder plates and pressurized by Teflon screws. **(b)** Glass chip substrates were modified with hydrophobic molecules not to leak liquid. **(c)** Design of glass chip. Top substrate has blood cell channel and bottom has plasma channel.

**RESULTS AND DISCUSSION**

The critical parameter to realize efficient filtering is a fluidic resistance of the porous membrane. Figure 3 shows a result of the resistance measurements for different pore sizes. Unexpectedly, all the resistances were 75 times higher than the catalog values, which might be due to a deformation of the membrane by pressurizing. Using these resistance, back pressure of each microchannel was calculated to perform the plasma separation.

Figure 4 is the result of plasma separation. A membrane with a pore size of 1.2 μm had the highest collection efficiency of 18%, although 3 μm showed a leak of red blood cells through the membrane. In addition, hemolysis of the filtered plasma was also investigated. Figure 5 shows hemoglobin concentration in the separated plasma for each pore size. For all pore sizes, the hemoglobin concentration

![Figure 3: Fluidic resistance of membrane.](image)

**Figure 3: Fluidic resistance of membrane.**

![Figure 4: Plasma collection efficiency.](image)

**Figure 4: Plasma collection efficiency.**
in the plasma was below the concentration that is acceptable for CRP ELISA (300 mg/mL). From these results, the membrane with 1.2 μm pores was used for the following experiments. Figure 5 shows the correlation of μELISA signal of the plasma separated by the device and centrifuged plasma. As a real sample, one drop of human whole blood (50 μl) was diluted 5 times by saline to avoid unwanted effects due to the high viscosity. CRP was added to the sample as an internal standard substance. The two signals were well accorded with each other, which revealed availability of the plasma separation method.

CONCLUSION
To conclude, a microfluidic plasma separation device was developed and applied to whole blood immunoassay. The separation device combining microfluidic effect and membrane filtering could separate 18% plasma from whole blood without severe hemolysis. Then, CRP in the separated plasma was successfully determined using μELISA, which enabled rapid analysis from one drop of whole blood.

REFERENCES

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