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Separation Method of Blood Constituents Using Dielectrophoresis and Flow-Induced Shear Force

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Abstract— **Platelet Rich Plasma (PRP) contains many cytokines for treatment skin diseases. The final goal of this study is to develop the dielectrophoretic PRP purification system enriching the platelets in a label-free manner from whole blood solution. In this study, we characterized dielectrophoretic properties of red blood cell, white blood cell, and platelet for the fundamental study. Moreover, purification of PRP was performed to eliminate red and white blood cells using dielectrophoretic and flow-induced shear force.**

I. Introduction

Platelets are the smallest cell in blood constituents, and they play a critical role in hemostasis and thrombosis [1]. They are frequently transfused to patients undergoing a wide variety of medical procedures including general surgery, treatment of trauma or skin disease patients [2]. Especially, injection of Platelet Rich Plasma is considered to be an effective approach to treat skin disease.

Centrifugation is the most widely used method of platelet purification. A number of protocols have been developed which include the platelet-rich plasma preparation (PRP), the buffy-coat preparation (BC), and apheresis [3]. Due to the fact that platelets become activated by mechanical shear stress, any separation step that requires high-speed centrifugation [4] results in significant loss [5]. Both PRP and BC methods have been reported to loose about 50% of the original number of platelets [3]. High-purity, low-stress platelet separation technology becomes increasingly important.

Dielectrophoresis (DEP) is one of the most promising approaches for separating microparticles and biological particles such as bacteria and blood cells [6]-[9]. DEP is a phenomenon caused under an applied non-uniform electric field inducing dipoles within a polarized cell in a buffer solution. This phenomenon can lead to a nonzero coulombic net force on the cell causing its movement. Cells can be moved by DEP forces to move toward high or low electric field regions, depending on the relative electric property of the cells in suspending medium [10]. Since the DEP force magnitude is scaled to the cubic power of cell size, the sorting of cells by size is studied widely [11].

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Final goal of this study is to develop PRP separation technology using dielectrophoresis (DEP) and flow control system. For the fundamental study, the dielectrophoretic properties of red blood cell, white blood cell, platelet were characterized in this study. Using these results, we established the PRP purification method to eliminate red blood cells and white blood cells using DEP force and flow-induced shear force.

II. Dielectrohoresis

For a spherical particle in a non-equal electric field, the time-averaged DEP force is generated on the particle as

$$\vec{F}_{DEP} = 2\pi r^3 \varepsilon_0 \varepsilon_m \,\mathrm{Re}\left[f_{CM}(\varpi)\right] \nabla E_{rms}^2 \qquad (1)$$

where r is the radius of micro particle, ε_0 is the permittivity of the vacuum, ε_m is the relative permittivity of the medium, $\mathrm{Re}[f_{CM}]$ is the real part of the Clausius-Mossoti (CM) factor, E_{rms} is the root mean square value of the electric field [10].

The CM factor is related to the magnitude and direction of the DEP force. If the CM factor is plus, the DEP force caused on particle is directed toward the region of high electric field intensity (p-DEP). Conversely, if the CM factor is minus, the DEP force is directed toward the low field intensity. The CM factor is shown as

$$f_{CM}(\varpi) = \frac{\varepsilon_p^* - \varepsilon_m^*}{\varepsilon_p^* + 2\varepsilon_m^*} \qquad (2)$$

where $\varepsilon^*_{\mathrm{p}}$ and $\varepsilon^*_{\mathrm{m}}$ are the complex permittivity of the micro-particle and the suspended medium. Each complex permittivity is defined as

$$\varepsilon^* = \varepsilon_0 \varepsilon - \frac{\mathrm{j}\sigma}{\varpi} \qquad (3)$$

where $\mathrm{j} = \sqrt{-1}$, ε is the relative permittivity of the particle or medium, σ is the electric conductivity and ω is the angular frequency of the applied AC electric field. This equation shows the dependency of CM factor on not only the electric property of the particle and medium but also on the frequency of the applied AC electric field. Therefore, DEP forces have possibility to separate cells based on their own electric properties that reflect cell functionality.

III. Materials and methods

A. Blood sample and isolation of each constituents

Red blood cell: Whole blood was extracted via venipuncture from adult donor at St. Marianna University School of Medicine. To prepare red blood cells (RBCs), the

whole blood was centrifuged and resuspended in 5% D-glucose solution. Just prior to the experiments, the RBCs were diluted 1:20000 by low-conductivity buffer (LC buffer; 10 mM HEPES, 0.1 mM CaCl2, and 59 mM D-glucose in sucrose solution) [12].

White blood cell: To prepare white blood cells (WBCs), the whole blood was mixed with dextran solution (20% v/v of whole blood) and left 30 min to fractionate the whole blood sample. Then, the upper layer containing the WBCs was centrifuged and resuspended in 5% D-glucose solution. Just prior to the experiments, the WBCs were diluted 1:2 by LC buffer.

Platelet Rich Plasma: PRP samples were prepared by a double-centrifugation procedure. At first, the whole blood was centrifuged to separate platelets containing layer (upper layer) and blood cells containing layer (lower layer). Next, the upper layer was centrifuged again to concentrate the platelets. Finally, the concentrated platelets were resuspended in 1 ml of 5% D-glucose solution. Just prior to the experiments, the PRP solution was diluted 1:2 by LC buffer.

B. *Fabrication of DEP chamber and experimental set-up*

To fabricate a transparent parallel-line-electrode array on slide glass, ITO (Indium-Tin-Oxide)-coated glass (Geomatec, Kanagawa, Japan) was used for transparent conductive substrate. The thickness of ITO-layer was 1500 A^{o} and resistance was 5 Ω/sq. The pattern of the electrodes was made using laser-etching techniques. The parallel-line micro-electrodes were designed to generate a highly non-equal electric field (Fig. 1). Each electrode line has a width of 20 μm and was spaced 100 μm apart.

The flow channel was made from silicon rubber sheet to establish rectangular volume (Fig. 1A). The dielectrophoresis chamber was formed by sandwiching the silicon rubber gasket between the parallel-line electrode array and a bare ITO-coated slide glass drilled with holes for fluidic inlet and outlet. The silicon rubber gasket determines chamber height. The resulting geometry of DEP chamber was 17.5-mm long from the inlet to electrodes, 5-mm wide, and 500-μm high (Fig.2). The length of the flow channel was determined to ensure whole blood cells were dropped on the electrode array.

A 1 ml-disposable syringe containing blood specimens was connected to the inlet of microchannel and the blood specimens was injected manually or using a twin-syringe pump (Harvard 33 Twin Syringe Pump, Harvard Apparatus, Holliston, MA). After the injection of sample solution, the characterizations of DEP properties of blood cells were performed.

An AC electric voltage was applied between the parallel-line electrodes and bare electrode, using a function generator (WF1974, NF, Kanagawa, Japan) and amplifier (BA4850, NF, Kanagawa, Japan), with monitoring the voltage by an oscilloscope (TDS1001B, Tektronix, Beaverton, OR) connected in parallel. The movements of the cells in the DEP chamber were observed, using a phase-contrast microscope (BX51, Olympus, Tokyo, Japan) with a digital video camera.

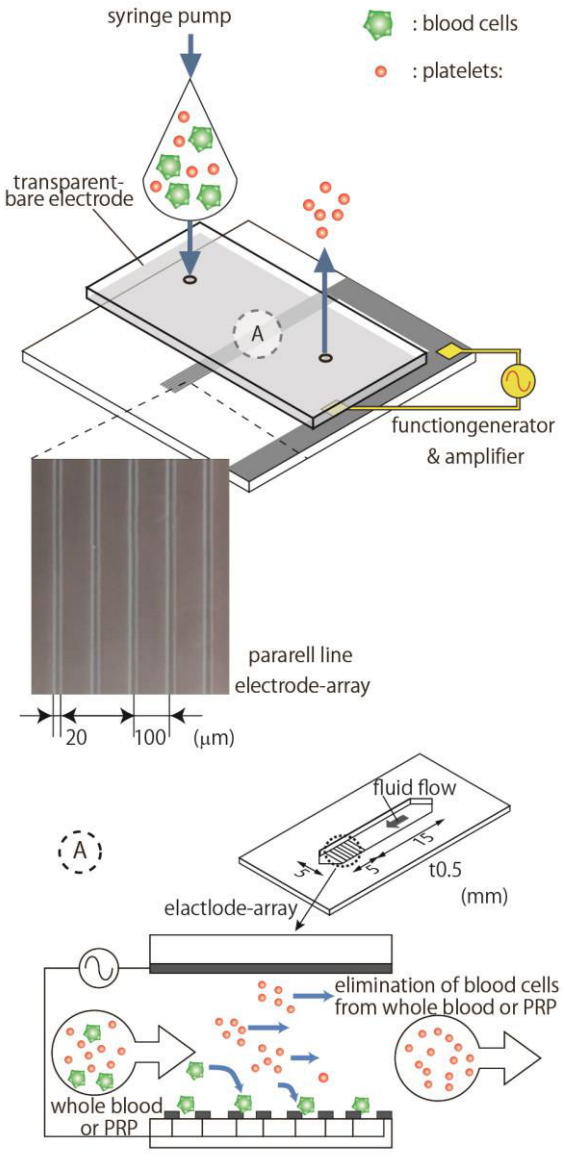

Figure 1. Schematic of dielectrophoretic chamber and desing of electorde-array.

C. *Characterization of DEP properties of Blood cells*

To characterize the frequency dependency of DEP properties of blood constituents, dielectrophoresis experiments were performed under various frequencies of AC voltage. During the experiments, the voltage was set to 20 $V_{p\text{-}p}$, and the frequency was varied from 10 kHz to 120 kHz. The movements of blood cells were recorded by the digital video camera equipped on microscope, and images were captured at 0 s and 180 s after imposing AC voltage for the analysis of DEP property.

The captured images were trimmed to analyze a region of 500 μm x 500 μm, and the number of cells on the electrodes (positive-DEP) and between the electrodes (negative-DEP) were counted respectively as shown in Fig. 2. The frequency dependency of DEP property was evaluated as

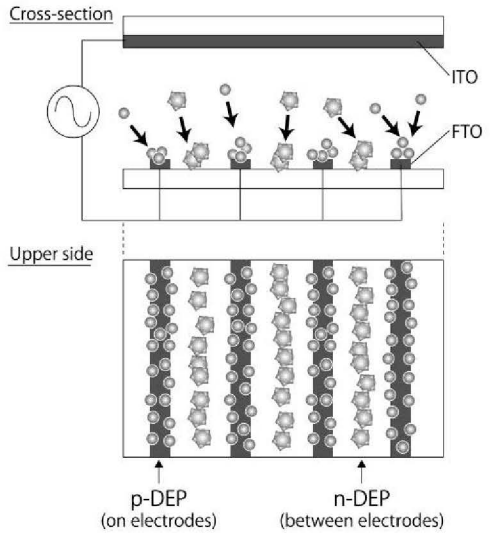

Figure 2. Evaluation of dielectrophoretic properties of blood cells.

$$N_P = n_P/(n_P + n_N),\ N_N = n_N/(n_P + n_N) \quad (1)$$

where n_P and n_N are the number of blood cells under positive- and negative-dielectrophoresis respectively. However, the platelets were only qualitatively evaluated because they were too small to count the number even under the phase contrast microscopy. ITO

D. Elimination of red and white blood cells from PRP solution using DEP force and flow-induced shear force

Plate Rich Plasma was prepared as described above. Though the PRP was purified by the double-centrifugation procedure, the RBCs and WBCs remained in the PRP solutions. Therefore, the DEP purification of PRP was assessed in this section. The principle of blood cell elimination from the PRP was shown in Fig. 3. The PRP solution was induced into the DEP chamber using a syringe pump and the AC voltage was imposed on the chamber to generate the DEP forces. The numbers of blood cells were counted at inlet and outlet of the chamber for evaluating the purification efficacy of the PRP solution. A 20 $V_{p\text{-}p}$, 150, 250, 500, and750 kHz sinusoidal AC voltage was applied via the function generator and amplifier, and monitored by the oscilloscope connected in parallel. During imposing the AC voltage, the PRP solution was pushed out at flow rate of 1.0 ml/min to cause fluid-induced shear stress 0.18 Pa. This shear stress was weak enough to avoid platelet activation. The number of blood cells in PRP were counted at inlet of the chamber and the numbers of those were also counted at outlet to evaluate the purification efficacy of purification.

IV. Results and Discussions

All blood constituents (red blood cells, white blood cells, and platelets) showed different dielectrophoretic response depending on the imposed frequency of ac voltage.

Fig. 4 shows the dielectrophoretic property of red blood cells (RBCs). The RBCs showed negative-DEP up to 80 kHz and ratio of cells for p-DEP increased dramatically between 80 and 100 kHz. Over 100 kHz, the RBCs exhibited the p-DEP behavior. Around a crossover frequency of dielectrophoresis, cells experienced the positive- or negative-DEP depending on their own electric properties. Therefore, the frequency where the cells experienced both positive- and negative-DEP equally was speculated to be crossover frequency. Therefore, the crossover frequency of RBCs was ranging from 80 to 100 kHz.

Fig. 5 shows the DEP behavior of white blood cells (WBCs). The WBCs showed negative-DEP up at 20 kHz and ratio of cells for p-DEP increased dramatically between 20 and 50 kHz. Over 50 kHz, the WBCs exhibited the p-DEP behavior. From this result, the crossover frequency of WBCs was ranging from 20 to 50 kHz.

Fig. 6 shows the photomicrograph of platelets under DEP at 75 and 100 kHz. The platelets showed negative-DEP up to 75 kHz, while negative- and positive-DEP at 100 kHz. Therefore, the cross over frequency was speculated to be around 100 kHz.

From the results of dielectrophoretic characterization of the blood constituents, RBCs and WBCs were discriminated by DEP phenomena alone. However, it was difficult to separate the platelets from RBCs and WBCs because RBCs and platelets showed similar crossover frequencies. Therefore, separating the platelets from RBCs and WBCs required a combination of DEP force (proportional to cubic power of cell diameter) and fluid-induced shear force (proportional to quadratic power of cell diameter).

Fig. 7 shows the number of RBCs and WBCs before and after the dielectrophoretic separation of platelets from the PRP solutions. Under all imposed frequencies, the number of blood cells decreased under the half of that before DEP purification. However, the number of platelets decreased to the 1/3 of initial number at 150 kHz, and under 1/10 of initial number at 250-750 kHz. For establishing the DEP purification system for PRP, the design of electrode array and flow channel of DEP chamber needs to be optimized with the platelets and blood cells.

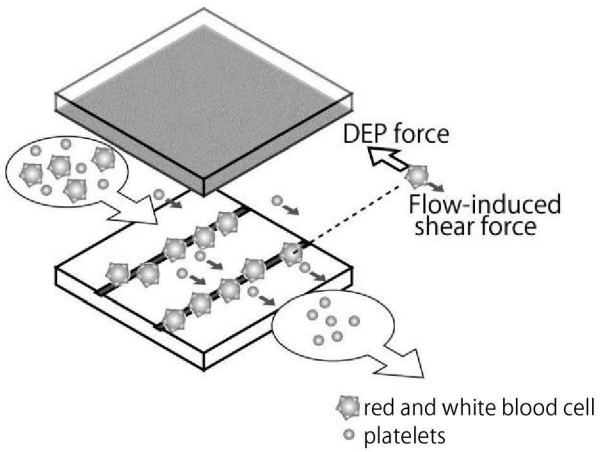

Figure 3. Elimination of blood cells from PTP solution by DEP and fluid-induced shear forces. .

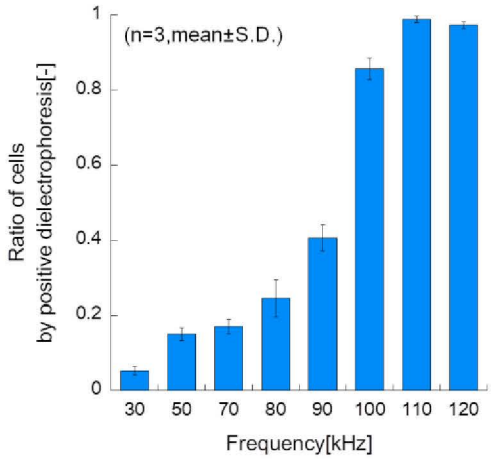

Figure 4. Ratio of RBCs experienced positeive-DEP.

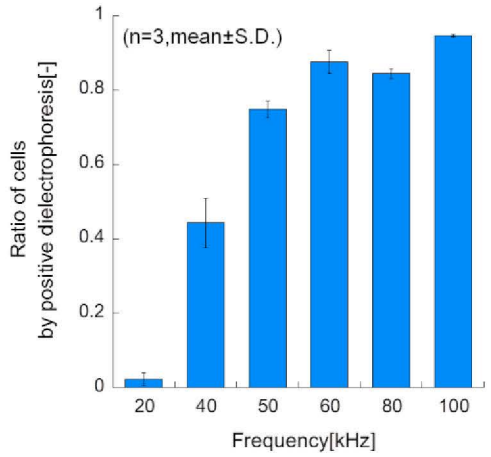

Figure 5. Ratio of WBCs experienced positive-DEP.

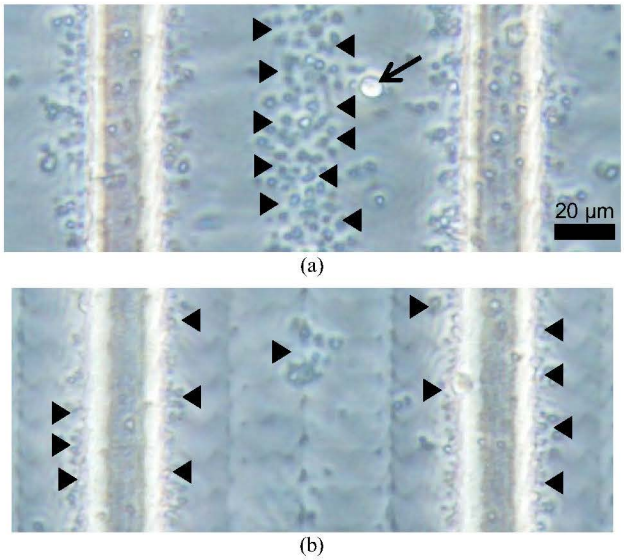

Figure 6. Photomicrograh of platerets in PRP solution under dielectrohpresis at 75 kHz (a) and 100 kHz (b). Vector arrow indicates a blood cell and arrowheads indicate platelets.

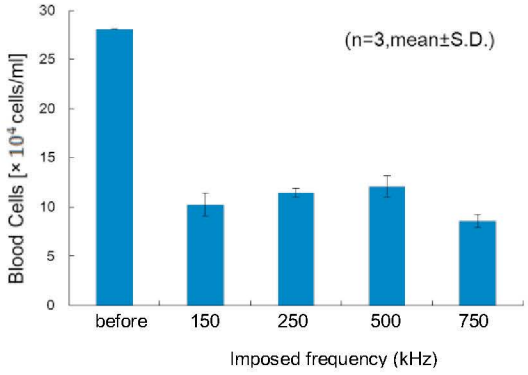

Figure 7. Number of blood cells in PRP solution before and after DEP purification experiments.

V. Conclusion

In this study, the dielectrophoretic properties of blood constituents (red and white blood cells, and platelets) were evaluated. From the evaluation, the red blood cells showed the different DEP behavior form that of white blood cells, the similar behavior to that of platelets.

Using the results of DEP characterization, the PRP solution was purified to eliminate the blood cells by DEP force and flow-induced shear force. The results exhibited the possibility of DEP purification system for PRP therapy.

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