Microchip 내부 혈액의 스페클 분석을 통한 적혈구 응집 측정

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Speckle analysis in a microchip for measurement of red blood cell aggregation

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Abstract : Increase of red blood cell (RBC) aggregability would significantly increase the vascular flow resistance contributing to peripheral vascular diseases. Precise measurement of RBC aggregation is important to understand the outbreak of peripheral vascular diseases. In this study, a simple analysis using a microchip is proposed to sensitively estimate RBC aggregation. Given that formation of RBC aggregates in the channel makes speckle patterns when the flow rate in the channel considerably decreases, the extent of RBC aggregation can be estimated by calculating a speckle area (A_{Speckle}) through a normalized autocovariance function. These experimental demonstrations support the notion that the proposed method is capable of effectively monitoring the variation of RBC aggregation.

1. Introduction

Blood is a concentrated suspension of red blood cells (RBCs), leukocytes, platelets, and protein macromolecules in plasma. RBCs are the most important component of blood because they occupy large volume concentration, as well as furnish tissues with oxygen and nitric oxide, and receive carbon dioxide in return. In plasma, RBCs form RBC aggregates as a reversible process. This phenomenon is interest in hemorheology, because the shear dependence of viscosity is mainly attributed to RBC aggregation. Specifically, large rouleaux formations under low shear condition lead to the elevation of blood viscosity. Increase of RBC aggregation has been considered to significantly increase vascular flow resistance. In the present study, a speckle analysis of blood flows caused by light scattering in a microchip is proposed to simply and precisely measure the extent of RBC aggregation.

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2. Methods

As shown in Fig. 1, the rectangular microchip (height = 50μm, width = 1mm, length = 10mm) was mounted on an optical microscope (Nikon, Tokyo, Japan) with 10× objective lens (NA of 0.25). According to decrease of injection profile from 1 to 0 mL/h, the blood samples were supplied into the microfluidic device by using a syringe pump (neMESYS, Centoni Gmbh, Germany) with a 1mL plastic syringe (BD; Becton Dickinson, Franklin Lakes, USA). A high-speed CMOS camera (FASTCAM SA 1.1, Photron Ltd., San Diego, USA) was used to acquire four consecutive images of blood flows at a frame rate of 5000 fps. An array-type, high-powered LED was employed as an illumination source.

Blood samples, collected through an abdominal aortic puncture of Sprague-Dawley (SD) rats, were anticoagulated using ethylenediaminetetraacetic acid (EDTA) dipotassium salt (1.5 mg of EDTA per 1

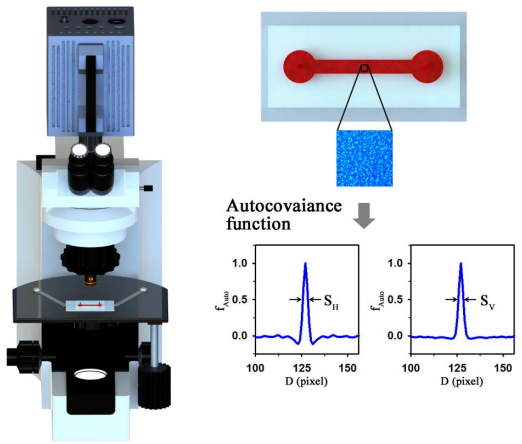

Fig. 1 Schematic diagram and procedure of speckle analysis.

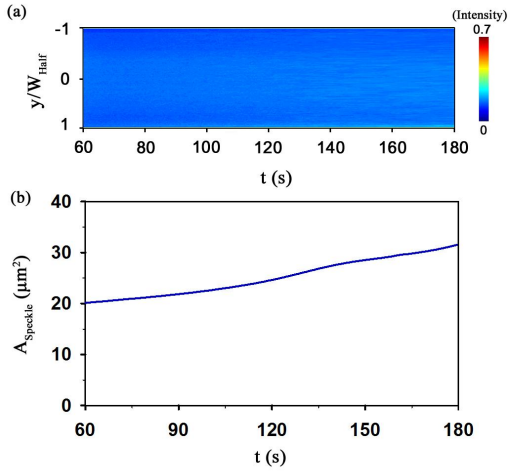

Fig. 2 Temporal variation of (a) blood speckle and (b) speckle area according to decrease of flow rate.

mL of blood). Hematocrit level of blood samples was precisely set to be 40 %.

3. Result and discussion

Right side of Fig. 1 shows procedure of speckle analysis based on the normalized autocovariance function. The intensity autocorrelation function is determined by the following equation;

$$C_I(\Delta x, \Delta y) = \frac{FT^{-1}[\,|FT[I(x,y)]^2|\,] - \langle I(x,y)\rangle^2}{\langle I(x,y)^2\rangle - \langle I(x,y)\rangle^2} \quad (1)$$

where $I(x,y)$ is the intensity value in the image plane (x,y). $\Delta x = x_1 - x_2$ and $\Delta y = y_1 - y_2$; (x_1,y_1) and (x_2,y_2) are two specific positions. FT indicates the Fourier transformation and the symbol $< >$ represents the spatial averaging over the image. $C_I(\Delta x,0)$ and $C_I(0,\Delta y)$ are the horizontal and vertical profiles of $C_I(\Delta x,\Delta y)$, respectively. By applying Eq. (1) to ROIs in the channel, the normalized autocovariance functions (f_{Auto}) along the horizontal and vertical directions are obtained. Then, the full width at half maximum of the calculated f_{Auto} provides the information on the representative size of the speckle pattern. Therefore, the average speckle area ($A_{Speckle}$) can be calculated by multiplying the horizontal and vertical speckle sizes.

As the flow rate decreases from 1 to 0 mL/h, the EA formation is promoted. Therefore, the blood image becomes to have clear and bright speckles (Fig. 2 (a)). As shown in Fig. 2(b), $A_{Speckle}$ gradually increases with the lapse of time. This result indicates that $A_{Speckle}$ can be used as a useful tool to evaluate variations of RBC aggregation in patients with various cardiovascular diseases.

Acknowledgments

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Reference

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