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Effects of red blood cell aggregates dissociation on the estimation of ultrasound speckle image velocimetry

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ABSTRACT

Ultrasound speckle image of blood is mainly attributed by red blood cells (RBCs) which tend to form RBC aggregates. RBC aggregates are separated into individual cells when the shear force is over a certain value. The dissociation of RBC aggregates has an influence on the performance of ultrasound speckle image velocimetry (SIV) technique in which a cross-correlation algorithm is applied to the speckle images to get the velocity field information. The present study aims to investigate the effect of the dissociation of RBC aggregates on the estimation quality of SIV technique. Ultrasound B-mode images were captured from the porcine blood circulating in a mock-up flow loop with varying flow rate. To verify the measurement performance of SIV technique, the centerline velocity measured by the SIV technique was compared with that measured by Doppler spectrograms. The dissociation of RBC aggregates was estimated by using decorrelation of speckle patterns in which the subsequent window was shifted as much as the speckle displacement to compensate decorrelation caused by in-plane loss of speckle patterns. The decorrelation of speckles is considerably increased according to shear rate. Its variations are different along the radial direction. Because the dissociation of RBC aggregates changes ultrasound speckles, the estimation quality of SIV technique is significantly correlated with the decorrelation of speckles. This degradation of measurement quality may be improved by increasing the data acquisition rate. This study would be useful for simultaneous measurement of hemodynamic and hemorheological information of blood flows using only speckle images.

1. Introduction

The shear thinning effect of blood viscosity is mainly attributed to the dynamic behavior of red blood cells (RBCs) [1]. RBCs can aggregate and form rouleaux or three-dimensional networks of RBC aggregates. These RBC aggregates can be broken by disaggregating forces. Excessive RBC aggregation is correlated with cardiovascular diseases [2,3], diabetes [4], deep venous thrombosis [5] and others [6]. To assess RBC aggregation, various techniques were introduced, for example, by monitoring electrical impedance [7], light [8] and ultrasound [9,10]. However, most of those techniques can only measure the degree of RBC aggregation under *in vitro* or *ex vivo* conditions. As an exception, it is possible for ultrasonic measurement technique to observe the formation of RBC aggregation under *in vivo* conditions [11], because acoustic waves propagate through internal organs or tissues. In addition, the ultrasound imaging system can get the spatial and temporal distributions of RBC aggregates. For these reasons, the ultrasound modality has been widely used to measure the biophysical properties of RBC aggregation under both *in vivo* [12] and *in vitro* [13] conditions. Many studies demonstrated that RBC aggregation in a steady flow is dependent on the hematocrit and plasma fibrinogen concentration of blood, turbulence level, shear rate, vessel-wall compliance and flow disturbance [14–16]. Among them, the shear rate has been regarded as a crucial hemodynamic factor in RBC aggregation.

Because blood flows in arteries of a living being are pulsatile, the flow velocity is periodically varied according to a cardiac cycle. To understand the hemorheological features in blood flow with large velocity variation, the cyclic variations of echogenicity have been investigated using *in vitro* and *in vivo* models [9,12,17,18]. The cyclic variations of RBC aggregation under pulsatile flow are affected by not only shear force but flow acceleration [19,20]. However, the contribution of each factor to the RBC aggregation is still remained unclear [21]. For more systematic analysis, the

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effect of the shear force on RBC aggregation should be defined as another parameter. In light of the fact that the rouleaux and aggregates are separated under a shear force [22,23], the threshold shear-stress [8,24] and the strength of RBC aggregates [25,26] were suggested for estimating dissociation of RBC aggregates. However, since these methods monitored the variation of backscattered light intensity in a microfluidic rheometry, it could not directly detect the spatial distribution of RBC aggregation under *in vivo* flows. In addition, the shear-stress should be separately measured.

By adopting a high-frequency ultrasound transducer, velocity fields in a human superficial vein could be measured using ultrasound speckle image velocimetry (SIV) technique [27]. This technique applies the cross-correlation algorithm to speckle images scattered by RBCs or RBC aggregates. Recently, it was found that heterogeneous intensity distribution in ultrasound speckle induced by RBC aggregation degrades the measurement accuracy of SIV technique. The degradation of measurement quality was improved by applying an image-enhancement technique (IET) [28]. Therefore, it is now possible to measure the velocity field with reasonable estimation by using the SIV method coupled with IET.

The dissociation of RBC aggregates can be estimated by analyzing their speckle patterns in consideration of the speckle displacement measured by the SIV method coupled with IET. However, significant dissociation of RBC aggregates may affect the measurement quality of the SIV technique, because the variations in speckle patterns are closely related with the measurement accuracy of speckle tracking methods [29]. The objective of this study is to investigate the relationship between the dissociation of RBC aggregates and the estimation quality of SIV technique.

2. Materials and methods

2.1. Ultrasound imaging system

A GE LOGIQ 700 expert system (GE, Milwaukee, WI, USA) with an M12L linear-array probe was employed in this study to obtain ultrasound images of porcine blood flows [13]. The transducer frequency was set to 13 MHz. Longitudinal B-mode images were recorded consecutively. To check the performance of SIV technique, the flow velocity in the tube center region was checked *in situ* by using ultrasound Doppler spectrograms. Doppler range gate was set to 1 mm at the center of the tube and the pulse repetition frequency (PRF) was 1000. The video images were converted into digital images by using a video-editing system at 30 frames per second. A total of 3300 B-mode images were used for simultaneous measurement of instantaneous echogenicity and velocity fields. The physical dimension of each pixel in B-mode images was 0.16 mm × 0.16 mm.

2.2. Blood preparation and flow condition

Experiments were performed in a mock-up flow loop circulating porcine blood which has the similar RBC aggregation features with human blood [15]. Fresh porcine blood was obtained from a local slaughterhouse and then anticoagulated with ethylenediaminetetraacetic acid (EDTA) dipotassium salt. To remove any flesh or clots, the blood was filtered through a paper filter with pores of 30 μm. RBCs and plasma were separated by centrifugation. The buffy layer composed of platelets and white blood cells was removed. The separated RBCs were remixed with the autologous plasma to be 40% hematocrit. The reconstituted blood was circulated through a rigid polystyrene tube (Nalgene, Rochester, NY, USA) with an inner diameter of 9.5 mm. The tube was submerged in a water basin.

Fig. 1 shows a schematic diagram of the experimental setup used in this study. The inlet of the polystyrene tube was connected to the upper reservoir, while the outlet was opened to the lower reservoir. Blood flow was produced through the pressure difference between the two reservoirs. The centerline velocity (U_c) was decreased from about 23 cm/s to 0 during the data acquisition. Entrance length from inlet of the straight tube to the measurement section was set to be 1 m. The minimum entry length required for a fully developed tube flow was about 0.3 m [30]. Therefore, the entrance length was long enough to ensure a fully developed laminar flow. The maximum Reynolds number is about 375 by using a blood viscosity of 5.83 mPa s recommended in the previous study [31].

2.3. Speckle image velocimetry (SIV)

Fig. 2 depicts the procedure of velocity field measurement using SIV technique based on a cross-correlation algorithm [28]. To increase the measurement quality of SIV method, contrast-limited adaptive histogram equalization (CLAHE) method, which is a kind of IETs, was applied to original B-mode images. CLAHE divides one image into contextual regions and applies the histogram equalization method to each region. It adopts a contrast enhancement limit to transform original images into a value within the display range [32]. The pixel value g with an intensity histogram of Rayleigh distribution is calculated by

$$g = g_{\min} + \left[2 \times (\alpha^2) \times \ln \frac{1}{1 - P(f)}\right]^{0.5}, \quad (1)$$

where $g_{\min}$ is the minimum pixel value in each contextual region, α is a distribution parameter, and $P(f)$ indicates the cumulative probability distribution. In this study, the full range of grayscale, contex-

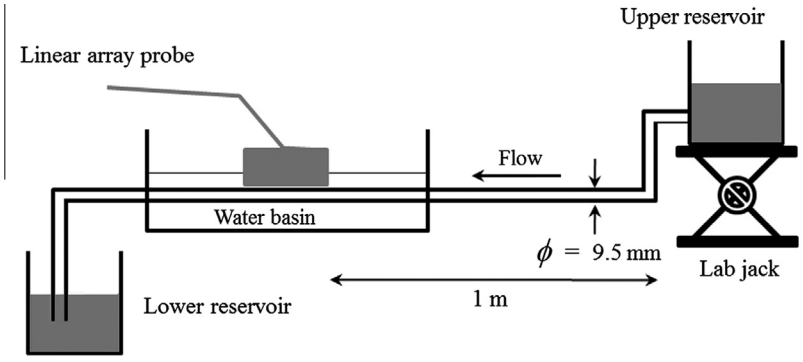

Fig. 1. Schematic diagram of the blood-flow loop for ultrasound imaging. ϕ denotes the inner diameter of polystyrene tube.