MEASUREMENT OF MULTI-RED BLOOD CELLS INTERACTIONS IN BLOOD FLOW BY CONFOCAL MICRO-PTV

R. Lima[1, 2], T. Ishikawa[1],H. Fujiwara[1], M. Takeda[1, 3], Y. IMAI[1],K. Tsubota[1], N. Matsuki[1, 4], S. WADA[5], T. Yamaguchi[1]

1) Dept. Bioeng. & Robotics, Grad. Sch. Eng., Tohoku Univ., 6-6-01 Aoba, 980-8579 Sendai, Japan.
2) Dept. Mechanical Tech., ESTiG, Braganca Polyt., C. Sta. Apolonia, 5301-857 Braganca, Portugal.
3) Div. Surgical Oncology, Grad. Sch. Medicine, Tohoku Univ., 2-1 Seiryo-machi, Aoba-ku, 980-8575 Sendai, Japan.
4) New Industry Hatchery Centre, Tohoku Univ., 6-6-01 Aoba, 980-857, Sendai, Japan.
5) Dept. Mechanical Science and Bioeng., Grad. Sch. Eng., Osaka Univ., Toyonaka, 560-8531 Osaka, Japan.

rui@pfsl.mech.tohoku.ac.jp

Introduction

In microcirculation the flow behavior of red blood cells (RBCs) plays a crucial role in many physiological and pathological phenomena. For instance, the interaction of RBCs in shear flow is believed to play an important role to the thrombogenesis process. Despite the relevance of this phenomenon on the blood mass transport, very little studies have been performed during the years, partly due to the absence of adequate visualization techniques able to obtain both direct and quantitative measurements on multi-RBCs motions in concentrated suspensions. Past studies on both individual and concentrated RBCs used conventional microscopes and/or ghost cells to obtain visible trace RBCs at high concentration suspension of blood cells [1, 2]. Recently, advances of confocal microscopy and consequent advantages over conventional microscopes have led to an emerging technique known as confocal micro-PIV [3, 4].

This paper presents the application of a confocal micro-PTV system to measure RBC-RBC hydrodynamic interactions in flowing blood.

Materials and Methods

Working fluids and microchannel: In this study we used dextran 40 (Dx40) containing about 20% (20Hct) of human red blood cells (RBCs). All blood samples were stored hermetical at 4ºC until the experiment was performed at controlled temperature of about 37ºC. The microchannel was a circular borosilicate glass (100 µm in diameter) .

Experimental set-up: The confocal micro-PTV system consists of an inverted microscope (IX71, Olympus) combined with a confocal scanning unit (CSU22, Yokogawa), a diode-pumped solid state (DPSS) laser (Laser Quantum Ltd) with an excitation wavelength of 532 nm and a high-speed camera (Phantom v7.1). The microchannel was placed on the stage of the microscope where the flow rate was kept constant by using a syringe pump. The confocal images were captured at a rate of 100 frames/s and then evaluated in Image J (NIH) [6] by using a manual tracking MTrackJ [7] plugin.

RBC radial displacement: The radial displacements (ΔR) of the tracked RBCs were determined by using a cumulative radial displacement, given by:

$$\Delta R = \sum_{i=0}^{n} \left| R_0 - R_i \right| \qquad (1)$$

where R_0 is the initial radial position and R_i is the cumulative radial displacement for a defined time interval.

Results and Discussion

Figure 1 shows the streamlines of two-RBC interactions around the plasma layer at Re = 0.007 ($\gamma \sim 16\ s^{-1}$). This figure shows clearly the radial disturbance effect due to the collision with a neighboring RBC.

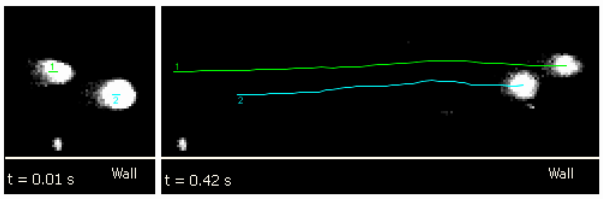

Figure 1 RBC interactions (40× objective lens).

Figure 2 shows the radial displacement of a RBC (RBCint) that have interacted with a neighboring RBC. Additionally, it is also shown the ΔR of a RBC (RBCnoInt) with any appreciable interaction at 3% Hct. These results show clearly the fluid-dynamical interactions effect on the motion of RBCs flowing in concentrated suspension of blood cells.

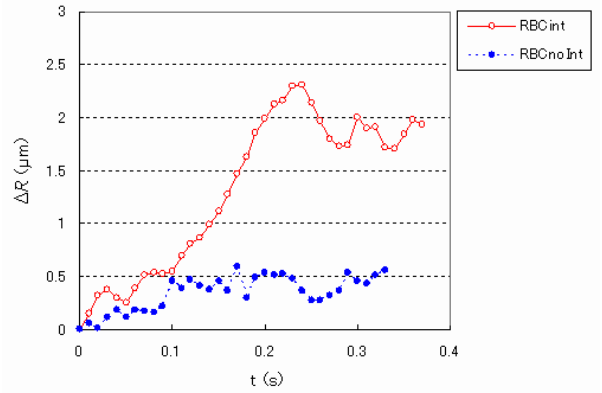

Figure 2 ΔR comparison between a RBC with interactions (20% Hct) and RBC with no interactions (3% Hct).

The present study provides both quantitative and qualitative evidence on RBC-RBC hydrodynamic interaction in flowing blood at Hct up to 20%. The measurements were possible due to the unique ability of the confocal systems to obtain thin in-focus planes. Hence, this confocal micro-PTV system can provide the paths of two or more RBCs cells interacting in the same focal plane. Such information is extremely important to elucidate the blood transport mechanisms and associated diseases such as thrombosis and atherosclerosis.

References

[1] Goldsmith, H., *Federation Proceedings*, **30**(5): 1578-1588, 1971.
[2] Goldsmith, H. and Marlow J., *Journal of Colloid and Interface Science*, **71**(2): 383-407, 1979.
[3] Lima, R., et al., *Meas. Sci. Tech.*, **17**: 797- 808, 2006.
[4] Lima, R., et al., *J. Biomech.*, (in press).
[5] Abramoff, M., et al., *Biophotonics International*, **11**(7): 36-42, 2004.
[6] Meijering, E., et al., *IEEE Signal Processing Magazine*, **23**(3): 46-53, 2006.