BLOOD FLOW ANALYSIS AND RED BLOOD CELL AGGREGATION INVESTIGATION

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ABSTRACT

This work aims to develop a method to quantitatively analyze red blood cell (RBC) aggregates under controlled flow conditions, based on image processing. The set up consists of RBC suspensions entrained by a second fluid in a 150x33 µm microchannel. The experiments are performed by varying the hematocrit (10, 15 & 20%) and the flow rate (Q=5 & 10 µl/hr). The flow is visualized using a high speed camera coupled to a micro Particle Image Velocimetry (µPIV) system. Videos obtained with the high speed camera are processed using a MATLAB program to detect RBC aggregates based on the images intensities. An average aggregate size has been determined for each of the shear rates and hematocrits. The aggregates are shown to be larger at low flow rates and high hematocrits.

INTRODUCTION

Red blood cells (RBC) play an important role in determining the rheological properties of blood. They are almost singularly responsible for the particular behavior of blood *in vitro* and *in vivo*. Under physiological conditions, RBC are able to deform and adapt to the smallest vessels in the body, the capillaries (4-10 µm), for gas exchange. At low shear rates, these cells have the unique ability to assemble and form aggregates (3D) thereby contributing to the non-Newtonian behaviour of blood. The formation of these aggregates can be altered by several factors such as the RBC membrane elasticity, suspending medium composition, the hematocrit of blood (volume of RBC in blood), vessel diameter and the shear rate [1-3].

RBC aggregates are typically seen at the macrocirculation level (>300 µm diameter). At this scale, blood is considered as a Newtonian fluid and a homogenous mixture. However, RBC aggregates are rarely seen at the capillary level (4-10 µm diameter) and are usually an indication of pathological conditions such as diabetes [4] and obesity. Other pathological conditions that could change RBC aggregation include inflammatory or infectious conditions, cardiovascular diseases such as hypertension or atherosclerosis and genetic disorders [5].

Therefore, understanding the RBC aggregation mechanism and analyzing these entities (by defining a relationship between the size of these aggregates and the flow conditions) could lead to understanding the microrheological behaviour of blood and hence to relating it to clinical applications. Nowadays, several methods are able to analyze aggregate formation that provide relevant information about blood behaviour by defining an aggregation index. However, these methods do not provide sufficient information to clearly define a relationship between the aggregate size and the flow conditions.

This work aims to develop a standard method to quantify RBC aggregates under controlled flow conditions using image processing. The blood velocity and shear rate are determined using a µPIV system while the flow is visualized using a high speed camera. The results obtained are then processed with a MATLAB code based on the image intensities in order to detect cell edges and determine aggregate size.

METHODOLOGY

Experimental set up

The experimental setup comprises a µPIV device (LaVision's MITAS) in order to visualize the flow and estimate the velocity field. This µPIV set up is composed of a CCD Image Intense camera (LaVision) with a resolution of 1376 x 1040 pixels and a pixel size of 6.45 x 6.45 µm, a NewWave Solo-II Nd:YAG laser (New Wave Research) emitting a wavelength of

λ = 532 nm and a MITAS inverted microscope (LaVision) with a 10x and a 40x lens. In order to control the position of the microchannel, the μPIV set up uses a moving stage, which is controlled by the Davis Imaging Software (LaVision). In order to determine the velocity field within the microchannel, tracer particles diluted at 1% in water ($d_{particle}$=0.86 μm, λ_{abs}=542 nm and $\lambda_{emission}$=612 nm) are introduced within the fluids, which illuminate when exposed to the appropriate wavelength. The fluids, contained in two 50 μl glass syringes (Hamilton), are pushed into a Polydimethylsiloxane (PDMS) microchannel at different flow rates using two pumps (Nexus3000, Chemyx and Picoplus, Harvard Apparatus). The motion of RBC in the microchannel is visualised using a high speed camera (Dalsa), with a resolution of 1024 x 1024 pixels and a pixel size of 10.6 x 10.6 μm, which is coupled with the LaVision device. The sensitivity of the device is 8 bits.

Fluid sample preparation

Three porcine blood samples (S, T, IV) are centrifuged three times at 3000 rpm for 10 mn in order to separate blood constituents. Blood plasma is collected from the first centrifugation to ensure minimum contamination with white blood cells and platelets. The red blood cells of each sample are then suspended in their original plasma at three different hematocrits (10%, 15%, 20%) to visualize and determine the effect of hematocrit on the aggregates. 3% of the tracer particle solution is added to each of the RBC-suspensions.

Microchannel

The microchannels are fabricated from PDMS using the standard photolithography methods. The Y-channel configuration is transferred to a photo-mask that is used to engrave the channel geometry on the wafer to create the mold. When exposed to UV light, the photo-resist (SU8-50 epoxy based negative photo-resist) hardens to form the channel mold. PDMS prepared from Slygard-184 (Dow-Corning). The channel is bonded to a glass slide using the oxygen plasma bonding method performed with the SP100 Anatech series (Anatech).

Flow visualization and data analysis

The μPIV technique used is particle based flow visualization that is able to provide a 2D velocity field within the microchannel. This technique requires tracer particles to be seeded within the fluids. The double pulsed CCD camera associated with the system captures sets of double frames (100 frames) at high speed separated by a *dt* dependent on the flow rate. The frames that are captured are synchronized with the laser pulse to illuminate the tracer particles that fluoresce under the microscope. The sets of frames are then cross-correlated and post-processed to obtain 2D velocity vectors. The double frames are discretized into interrogation windows with a size set by the user. The choice of the interrogation windows is crucial since it should take into consideration the full velocity distribution. Therefore, the window size should be proportional to the displacement of the particles. For each correlation window, a correlation map is plotted to determine the velocity vector by relating the highest point of this correlation map. The results are then averaged in time and space to obtain the experimental velocity profiles. The averaging technique is used in order to reduce the effect of the Brownian motion of the particles and reduce the experimental error.

Video processing and aggregation analysis

Aggregate detection is performed using a MATLAB program based on the pixel intensities of the images. The methodology is detailed in Fig. 1. The frames of the captured videos are averaged for background subtraction, which allows a better image quality. The result is then converted into a binary image to be dilated. Each RBC in the image is approximated as a disk with predefined radius chosen according to the lens used for the video capture. The cells are then detected by determining the neighboring objects in the binary image. These cells are then labeled and the image is converted into a red-green-blue (RGB) image for better visualization. The aggregate size in pixels can thus be determined based on the RBC size. Using this methodology, we can obtain the distribution of the percentage of RBC within each aggregate as a function of the aggregate size.

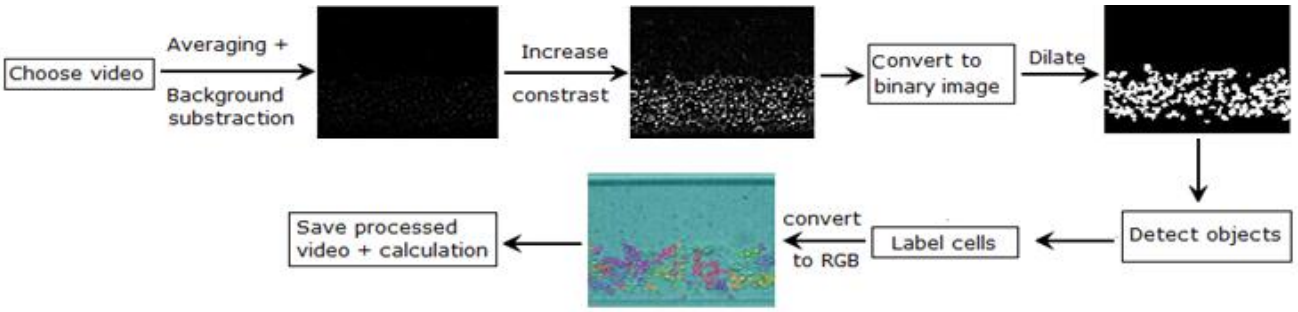


Figure 1: Flow chart of the MATLAB program

RESULTS AND DISCUSSION

RBC-suspensions, with three different hematocrits, are tested in the Y-microchannel configurations at two different flow rates. The corresponding shear rates determined using the μPIV set up are shown in Table 1.

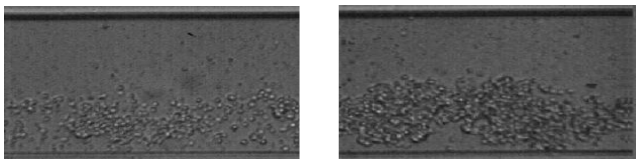
Table 1: Shear rate values for different cases

Sample	H	Flow rate	Shear rate
Blood S	10%	10 μl/hr	7.35 s^{-1}
Blood S	10%	5 μl/hr	5.18 s^{-1}
Blood T	10%	10 μl/hr	6.55 s^{-1}
Blood T	10%	5 μl/hr	5.21 s^{-1}
Blood IV	15%	10 μl/hr	6.96 s^{-1}
Blood IV	15%	5 μl/hr	4.06 s^{-1}
Blood S	20%	10 μl/hr	12.70 s^{-1}
Blood S	20%	5 μl/hr	5.12 s^{-1}

Frames of the videos captured with the high speed camera are shown in Fig. 2 for blood sample T suspended at 10% H flowing with Q=10 and 5 μl/hr respectively. It is expected, as seen in Fig. 2, that larger aggregates result from lower flow rates and hence shear rates. We can therefore extract the distribution of the aggregate size in the sample as well as the number of RBC in each aggregate for the different RBC-suspensions at 10, 15 and 20% H, as shown in Figs. 3, 4 and 5 respectively.

RBC-suspensions with 10% H

Fig. 3 shows the distribution of the aggregate size in the blood samples suspended at 10% H. We notice that for higher flow rates



(a) (b)

Figure 2: Blood sample T suspended at 10% H flowing with (a) Q=10 μL/hr and (b) Q=5 μL/hr

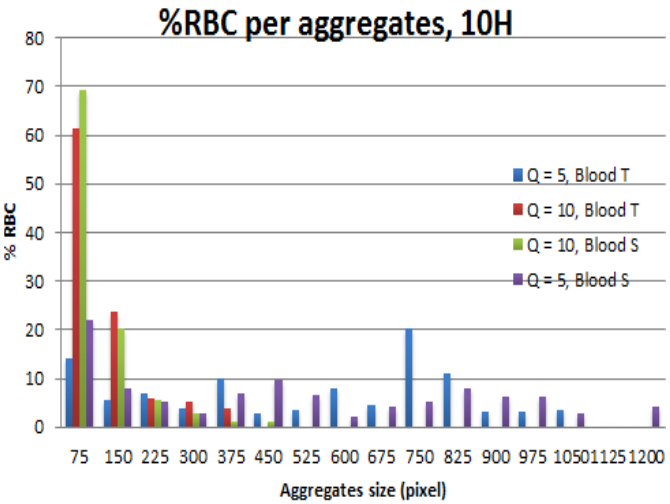


Figure 3: Aggregate size distribution for blood suspended at 10% H, flowing at 10 and 5 μL/hr

the aggregate size varies between 75 and 450 pixels while 60 to 70% of RBC are found in those aggregates. For lower flow rates the aggregate sizes are larger and reach about 1200 pixels. The distribution of the RBC in this case is evenly spread where 10 to 20 % of the RBC are found in the different aggregate sizes.

RBC-suspensions with 15% H

In order to perform this test, the lens of the microscope was changed from 10x, as in the previous tests, to 40x lens, hence increasing

the precision. However, due to the high numerical aperture, the contrast between the background and the cells was not optimal. Therefore, it was difficult to identify the cells and thus the aggregates. This is seen through

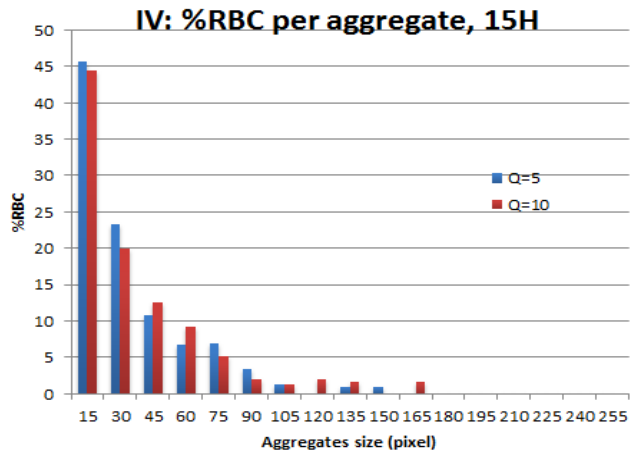


Figure 4: Aggregate size distribution for blood suspended at 15% H, flowing at 10 and 5 µL/hr

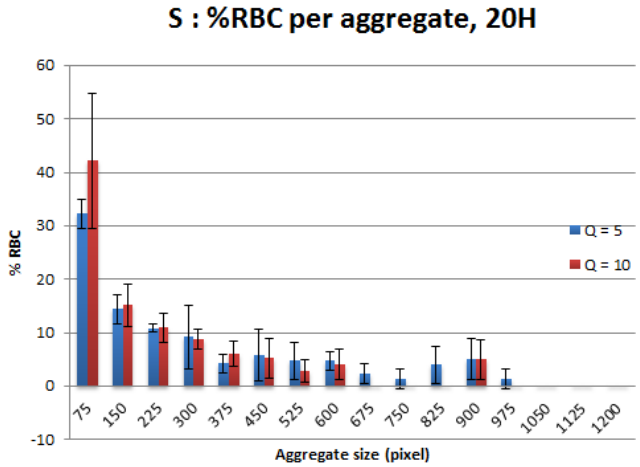


Figure 5: Aggregate size distribution for blood suspended at 20% H, flowing at 10 and 5 µL/hr. Standard deviation for averaging is represented by the error bars.

Fig. 4, which describes aggregate size distribution for blood suspended at 15% H. In fact, the distribution for both flow rates is similar, which is not expected according to previous results with the samples at 10% H.

RBC-suspensions with 20% H

The tests with the RBC suspended at 20% H were performed again with the 10x lens. Due to the large number of RBC in the blood layer, the aggregates for both flow rates are difficult to detect based on image processing. Further investigation is needed. The results show that the distribution of aggregate size (Fig. 5) is very similar for both flow rates which does not provide enough information to discuss this specific case. We notice however that larger aggregates are seen when Q=5 µl/hr, with sizes up to 975 pixels. The error bars, shown in Fig. 5, represent the standard deviation for both flow rates which indicates that the different tests performed with the 20% H blood suspensions are consistent. Similar results were found for suspensions at 10% and 15% H.

CONCLUSION AND FUTURE WORK

RBC-suspensions were successfully tested with different flow rates and hematocrit under controlled flow conditions for a qualitative and quantitative RBC aggregate analysis. It was shown that larger aggregates were present when the RBC are suspended at low hematocrit and flowing at low flow rates. For higher flow rates, smaller aggregates of up to 750 pixels are present. Due to the objective lens used and the large number of RBC in the blood layer, detection of aggregates for the 15% and 20% H suspensions was difficult. Further investigation is required, where more tests would be performed with a higher objective lens magnification without obstructing the light intensity. Future work aims to further develop the method to obtain a relationship between the shear rates, measured using the µPIV system, and the aggregate sizes.

REFERENCES

[1] O. K. Baskurt. *Handbook of Hemorheology and Hemodynamics*. IOS Press, Netherlands, 2007.

[2] R. Fahraeus and T. Lindqvist. "The viscosity of the blood in narrow capillary tubes", *Am. J. Physiol. – Legacy Content*, vol. 96, pp. 562–568, 1931.

[3] H. L. Goldsmith, G. R. Cokelet, and P. Gaehtgens. "R. Fahraeus: Evolution of his concepts in cardiovascular physiology", *Am. J. Physiol*, vol. 257, pp. H1005–1015, 1989.

[4] O. Baskurt, B. Neu, and H. J. Meiselman. *Red Blood Cell Aggregation*. Taylor and Francis, Florida, 2011.

[5] Y. I. Cho, M. P. Mooney, and D. J. Cho, "Hemorheological disorders in diabetes mellitus", *J of Diabetes Sci Technol*, vol. 2, pp. 1130–1138, 2008.