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Red blood cells radial dispersion in blood flowing through microchannels: The role of temperature

Diana Pinho [a,b], Raquel O. Rodrigues [a,c], Vera Faustino [d], Tomoko Yaginuma [a], José Exposto [a], Rui Lima [a,b,e,*]

[a] Polytechnic Institute of Bragança, ESTiG/IPB, C. Sta. Apolónia, 5301-857 Bragança, Portugal
[b] CEFT, Faculdade de Engenharia da Universidade do Porto (FEUP), R. Dr. Roberto Frias, 4200-465 Porto, Portugal
[c] LCM – Laboratory of Catalysis and Materials – Associate Laboratory LSRE/LCM, Faculdade de Engenharia, da Universidade do Porto (FEUP), R. Dr. Roberto Frias, 4200-465 Porto, Portugal
[d] Unidade de Investigação em Microssistemas Eletromecânicos (MEMS), Campus de Azurém, 4800-058 Guimarães, Portugal
[e] MEtRiCS, DME, School of Engineering, University of Minho, Campus de Azurém, 4800-058 Guimarães, Portugal

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ABSTRACT

The behavior of suspensions of individual blood cells, such as red blood cells (RBCs), flowing through microvessels and microfluidic systems depend strongly on the hematocrit (Hct), microvessel topology and cell properties. Although it is well known that blood rheological properties are temperature dependent, to the best of our knowledge no work has studied the role of the temperature on the RBCs dispersion. A powerful way to investigate this latter effect is through a high-speed video microscopy system, which provides detailed flow measurements of each individual RBC. Hence, the effect of temperature on the RBCs dispersion flowing through a 100 μm glass capillary was examined by means of a confocal micro-PTV system. Hundreds of labeled RBCs were tracked at moderate Hct (12%) and at four different temperatures, i.e., 25 °C, 32 °C, 37 °C and 42 °C. The results yielded an enhancement of the RBCs diffusion as the temperature increases. Hence, our findings show that RBCs radial dispersion is temperature dependent and as a result the temperature should not be ignored in future blood flow studies. We believe that this finding is important for a better understanding of blood mass transport mechanisms under both physiological and pathological conditions.

1. Introduction

Human blood is a complex physiological fluid that consists of deformable blood cells suspended in plasma containing mostly water. It is well known that red blood cells (RBCs) are the most abundant type of cells in whole blood, with a concentration around 45% in human adults. Due the high RBC concentration, it is believed that rheological properties of whole blood are mainly determined by the presence of the RBCs within the flow. Hence, blood flow behavior in microcirculation has long been a hot topic of interest by the biomedical research community with an extensive history of experimental investigations (Chien et al., 1984; Goldsmith and Turitto, 1986; Lima et al., 2012; Garcia et al., 2012; Wong et al., 2012; Pinho et al., 2013b; Yaginuma et al., 2013; Faustino et al., 2014; Rodrigues et al., 2015).

In the last century, experimental blood flow has been measured by several measurements techniques such as: double-slit photometric (Nash and Meiselman, 1983), laser-Doppler anemometer (Cochrane et al., 1981; Uijttewaal et al., 1994). These techniques used direct photometric information obtained without image information. However, most successful techniques were the video-based methods (Goldsmith, 1971; Goldsmith and Marlow, 1979; Parthasarathi et al., 1999), where video-microscopy images were recorded in order to be analyzed off-line by using image processing techniques. Although the past results have been encouraging, detailed studies on blood flow behavior at a microscopic level have been limited by several factors such as poor spatial resolution, difficulty to obtain accurate measurements at such small scales, optical errors arisen from walls, high concentration of blood cells, and difficulty in visualization of the results due to insufficient computing power and absence of reliable image analysis techniques. However, recently due to advances in computers, optics, and digital image processing techniques, it has become possible to combine a particle image velocimetry (PIV) system with a spinning

* Corresponding author at: Mechanical Eng. Dep., School of Engineering, University of Minho, Campus de Azurém, 4800-058 Guimarães, Portugal. Tel.: +351 253510233.
E-mail address: rl@dem.uminho.pt (R. Lima).

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disk confocal microscope and consequently, improve both spatial and temporal resolution (Lima et al., 2008b, 2006). Due to its spatial filtering technique and multiple point light illumination system, this technique known as confocal micro-PIV, has become accepted as a reliable method for measuring blood velocity profiles with hematocrits (Hcts) up to 9% (Lima et al., 2007). For higher Hcts, the light absorbed by the RBCs contributes to diminish the concentration of tracer particles in the acquired confocal images and consequently generates spurious errors in the velocity fields. Hence, for high concentration of RBCs Lima and his colleagues have developed a new approach, known as confocal micro-PTV system (Lima et al., 2009a), able to obtain both qualitative and quantitative measurements in flowing blood at concentrated suspensions up to 35% Hct. By using this confocal system Lima et al. (2008a) have determined the RBCs radial dispersion coefficient (D_{yy}) in the middle plane of 50 μm and 100 μm glass capillaries at low Reynolds number (Lima et al., 2008a). The results demonstrated that RBCs D_{yy} tends to increase with the Hct and decrease with the diameter of the capillary. Similar results were also found by performing measurements in circular polydimethysiloxane (PDMS) microchannels (Lima et al., 2009b). More recently, Saadatmand et al. (2011) have studied the dispersion of fluid particles in a concentrated suspension of healthy RBCs by using confocal micro-PTV system. Results from this study yielded significant enhancement of the particle diffusion due to a micron-scale flow-field generated by the motions of the neighborhood RBCs. While the effect of Hct on the dispersion of RBCs and fluid particles has been widely investigated, according to our knowledge studies on the effect of temperature on the RBCs dispersion have never been performed.

A recent study performed by Quinn et al. (2011) has suggested that individual RBCs flowing in narrow microchannels become highly sensitive to changes of temperature. Thus, the effect of temperature on the RBCs dispersion in a concentrated suspension of healthy RBCs needs to be clarified. In this study, labeled RBCs in concentrated suspensions of healthy RBCs flowing in a glass capillary was investigated for four different temperatures, i.e., 25 °C, 32 °C, 37 °C and 42 °C (measured temperature ± 1 °C). By using a confocal micro-PTV system, we were able to track RBC motions with an Hct around 12%. We discuss both effect of temperature and RBCs radial position on RBC radial dispersion.

2. Materials and methods

2.1. Working fluids, experimental setup, and labeled RBC tracking

2.1.1. Working fluids and RBC labeling

The present study used Dextran 40 (Dx-40; Otsuka Medicine, Tokyo, Japan) containing 12 ± 2% (12 Hct) of human RBCs as a working fluid. The Hct corresponded to the feed reservoir Hct and was measured using a hematocrit centrifuge (Kubota 3220; Kubota Corp., Osaka, Japan) immediately before each experiment.

The RBCs were fluorescently labeled with a lipophilic carbocyanine derivative dye, chloromethylbenzamido (CM-DiI, C-7000; Molecular Probes, Eugene, OR, USA). More details on the labeling procedure can be found elsewhere (Lima et al., 2009a).

2.1.2. Microchannels, experimental setup and temperature control

In this study, 100 μm borosilicate glass microchannels fabricated by Vitrocom (Mountain Lakes, NJ, USA) were used. The capillary was mounted on a slide glass with a thickness of 80 ± 20 μm and was immersed in glycerol to minimize the refraction from the walls.

In order to trace the RBCs, we used the confocal micro-PTV system which consists of an inverted microscope (IX71; Olympus, Japan) combined with a confocal scanning unit (CSU22; Yokogawa, Japan), a diode-pumped solid-state (DPSS) laser (Laser Quantum, UK) and a high-speed camera (Phantom v7.1; Vision Research, USA). The glass capillaries were placed on the stage of an inverted microscope, and a constant pressure-driven flow was established (Re ~0.007) with a syringe pump (KD Scientific, Holliston, MA, USA). By using a thermal plate controller (Tokai Hit, Shizuoka, Japan) and a box made by expanded polystyrene, the temperature surrounding the capillary was maintained at 25 ± 1 °C, 32 ± 1 °C, 37 ± 1 °C and 42 ± 1 °C. The experimental temperatures were selected considering several physiological temperatures, such as 37 °C – the normal body temperature in humans, 42 °C – a severe fever temperature and 32 °C – a hypothermia body temperature. In addition, the room temperature (25 °C), which was often used in many past experimental blood flow studies, was also considered. A schematic view of the experimental set-up can be seen in Fig. 1.

2.1.3. Tracking of labeled RBCs

The images were taken with a 40× objective lens with a numerical aperture (NA) equal to 0.9. The confocal images were captured in the middle of the capillary with a resolution of 640 × 480 pixels at a rate of 100 frames/s and an exposure time of 9.4 ms. A manual tracking plug-in (MTrackJ) (Meijering et al., 2012; Pinho et al., 2013a) of the image handling software ImageJ (NIH, USA) (Abramoff et al., 2004) was used to track the labeled RBCs. Using this plug-in, the bright centroid of each

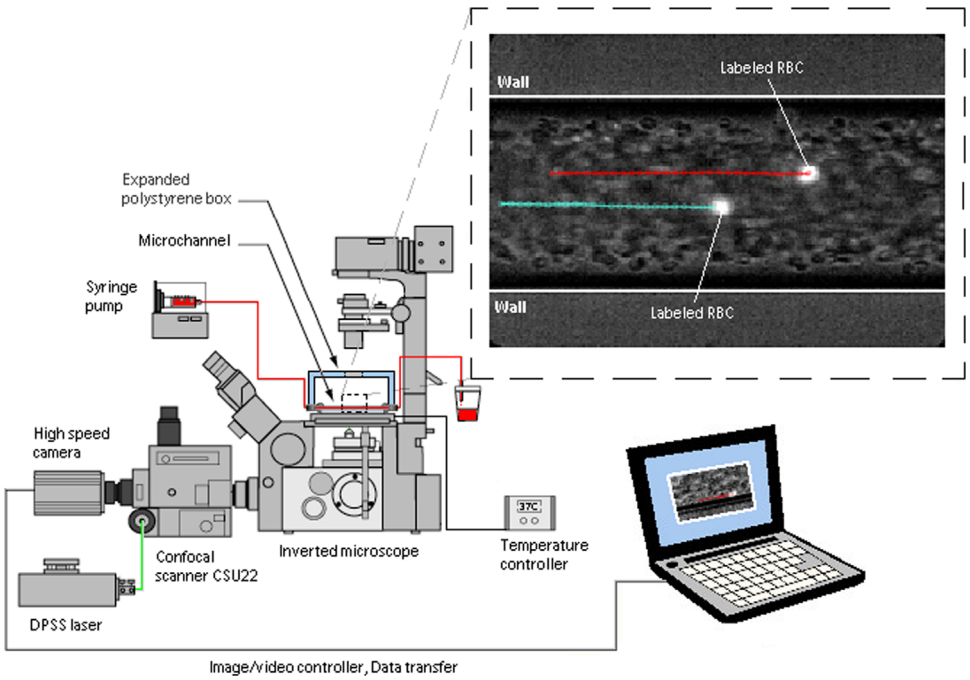

Fig. 1. Schematic diagram showing the confocal micro-PTV system used to measure the trajectories of each individual RBC flowing through the microchannel.

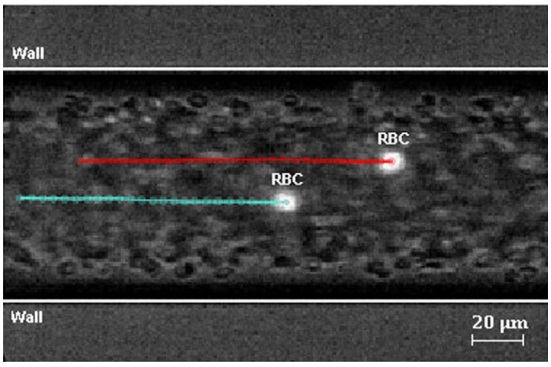

Fig. 2. Labeled RBC trajectories for 12% Hct. The non-labeled RBCs are observed as dark-gray rings, whereas the labeled RBCs are observed as bright dots.

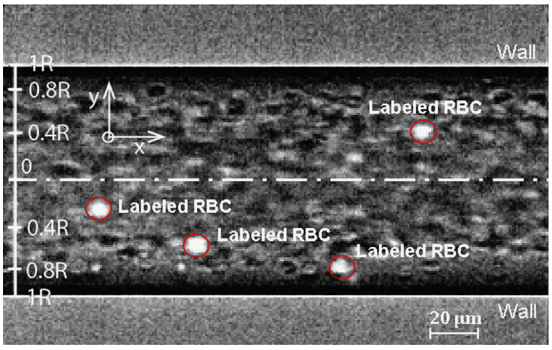

Fig. 3. Coordinate system in the middle plane of a 100-μm glass capillary. The image contains both halogen and laser light, which enables visualization of both labeled (bright dots) and non-labeled (dark-gray rings) RBCs.

selected RBC was automatically computed through successive images. The centroid x and y coordinate data for the individual positions of each RBC were then processed by a custom made ImageJ plug-in (DispersionCoefficient) to calculate radial dispersion coefficient, using immediate results obtained by the previous plug-in. Fig. 2 shows a typical view of labeled RBC trajectories tracked by the plug-in MTrackJ.

2.1.4. Rheological measurements

The viscosities of the working fluid (12% Hct) were measured at 25 °C, 32 °C, 37 °C and 42 °C by means of a stress control rheometer (Malvern/Bohlin CVO) with a cone-plate geometry (60 mm in diameter, 1° cone angle and 0.03 mm gap). The viscosity curves were obtained in a range of shears rate from 1 to 3000 s^{-1}. At least three replicas with fresh samples in each measurement were made in order to corroborate the reproducibility.

2.2. RBC radial dispersion coefficient

In the present study, the radial dispersion coefficient (D_{yy}) of individual RBCs measured at different temperature was calculated by using the ImageJ plug-in "DispersionCoefficient". The D_{yy} calculated in this plug-in is given by

$$D_{yy}(t) = \frac{1}{N}\sum_{i=1}^{N}\frac{\left\langle (R_{i,y}(t) - R_{i,y}(0))^2 \right\rangle}{2t}, \quad (1)$$

where $\left\langle (R_{i,y}(t) - R_{i,y}(0))^2 \right\rangle$ is the mean square radial displacement of the RBCs in the time duration t and N is the number of tracked RBCs. For the current study, more than 200 RBCs were measured.

3. Results and discussion

3.1. RBCs flowing at various regions in the 100 μm capillary

Fig. 3 shows the labeled RBCs flowing at various regions in the center plane of 100 μm capillary with 12% Hct (Re ~0.007) and temperature of 37 ± 1 °C. The labeled RBCs appeared as bright

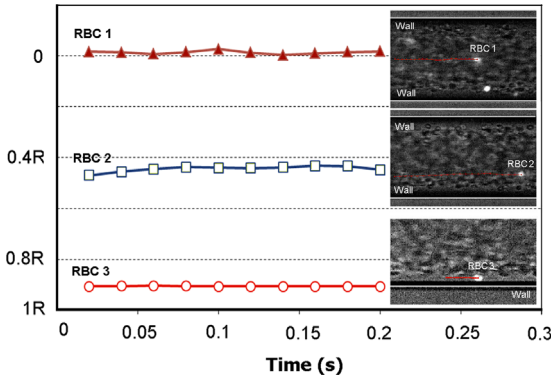

Fig. 4. Instantaneous transversal displacement of three representative RBCs (RBC 1, RBC 2 and RBC 3) at different radial positions: 0R–0.4R (RBC 1), 0.4R–0.8R (RBC 2), 0.8R–1 (RBC 3), for a temperature of 37 °C and Re ~0.007.

circular shapes among the other greyish non-labeled RBCs. The regions were divided into three sections (0–0.4R, 0.4R–0.8R and 0.8R–1R, where R is the radius of the capillary) and for each region RBC trajectories were measured.

3.2. Motion of individual RBCs at different radial positions

By using a confocal micro-PTV system, trajectories of individual RBCs were measured at Re ~0.007. Three representative examples for a temperature of 37 °C are presented below. Fig. 4 illustrates the instantaneous transversal displacement of three RBCs (RBC 1, RBC 2 and RBC 3) at different radial positions for a temperature of 37 °C. The transversal displacement, Ry (t), is the y position normal to the direction of the flow in a time interval, t (s).

These qualitative results show that for the case of a RBC rolling on the wall (RBC 1) the transversal oscillation is close to zero, whereas RBCs flowing within a crowded environment (RBC 2 and RBC 3) experience much greater fluctuation in their trajectories. Thus, the degree of the dispersion is dependent on the RBC radial position.

3.3. Radial dispersion coefficient by radial positions

Next, we have examined the D_{yy} tendency for each radial region (cf. Fig. 5). For all the temperature cases, the highest D_{yy} occurred in the region 0.4R–0.8R, which can be considered as the edge of the cell-rich center core. In this region, the motion of the flowing RBCs are more chaotic than the other regions, especially for the higher temperatures, 37 °C and 42 °C, since the value of D_{yy} keeps increasing with the time. We believe that the undulating RBC trajectories generated at the edge of the cell core region promote a large number of multibody collisions, which eventually lead to continuous flow disturbances at a microscopic level. Moreover, most RBCs flowing within the cell-free layer (CFL) tend to flow towards the center of the microchannel; however this tendency is inverted by the RBC core region and consequently enhances interactions between neighboring RBCs. This high D_{yy} may play an important role in the blood mass transport in microcirculation as several cells and proteins are transported into tissues. In contrast, in the region 0.8R–1R, the motion of the RBCs were very restricted. The region contains a CFL so that the number of the cells in this area was extremely low. Generally, it was observed that almost all the RBCs were flowing adjacent to the wall with a rolling motion. Moreover, when a RBC was rolling on the surface, most interactions did not promote a significant increase in the D_{yy}, indicating that the lubrication force between the RBC and the wall is greater than the hemodynamic force caused by a neighboring RBC collision. Last, but not least in the region 0R–0.4R have shown that the RBC D_{yy} is always in between the cases described above. Although at

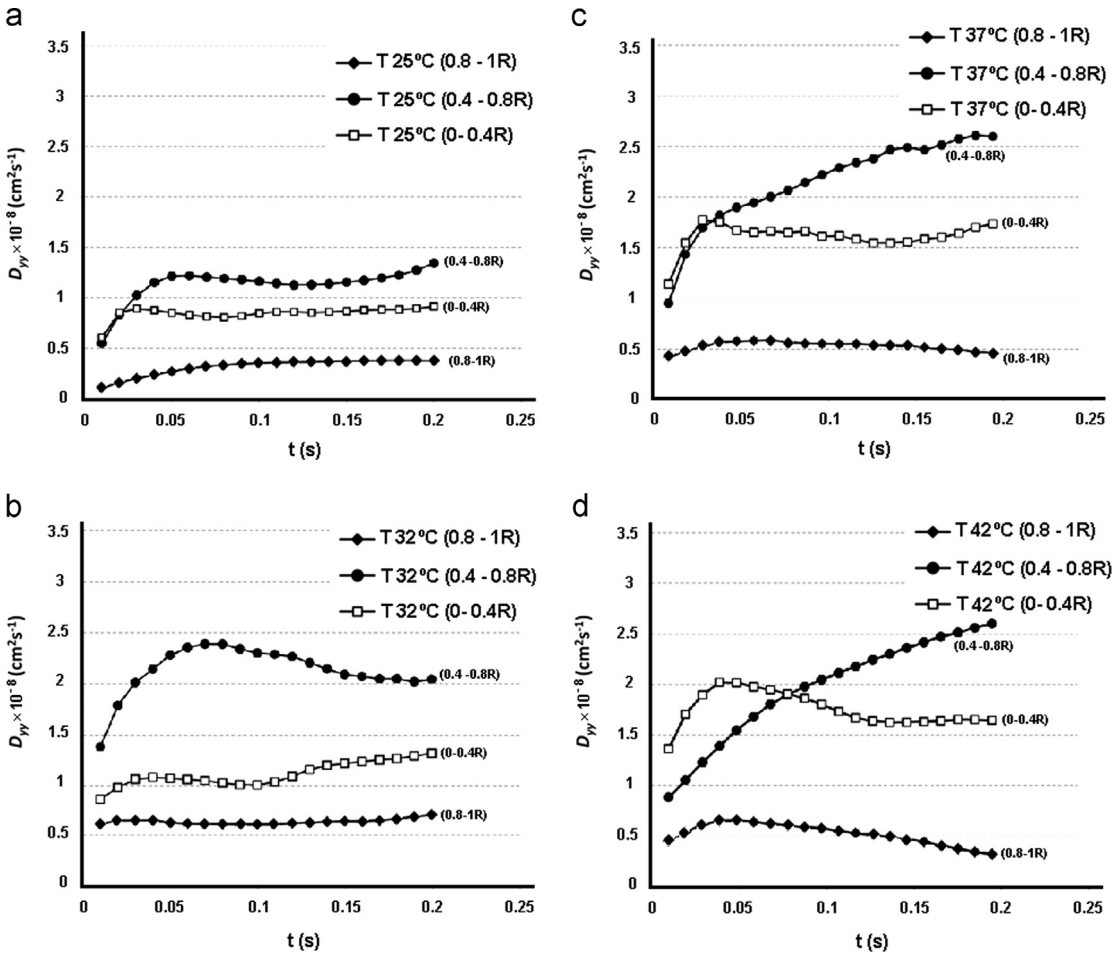

Fig. 5. RBC radial dispersion coefficient (D_{yy}) at different radial positions: 0R–0.4R, 0.4R–0.8R, 0.8R–1R and for different temperatures (a) 25 °C; (b) 32 °C; (c) 37 °C and (d) 42 °C.

this particular region there was the highest local concentration of RBC the D_{yy} was not the highest. We believe that the main reason for this moderate D_{yy} is because high concentrations of cells limit the amplitude of the RBC's radial motions. This latter result is consistent with other previous research performed by Goldsmith and Marlow (1979), Goldsmith and Turitto (1986) and Lima et al. (2008a).

3.4. Radial dispersion coefficient at different temperatures

The results of dispersion coefficient of tracked RBCs in a 100 μm capillary at different temperatures are shown in Fig. 6. We could see a tendency that the D_{yy} reached constant values around t=0.05 s for all the temperature cases except for the tested temperature of 42 °C, where the D_{yy} kept a slight increase even after leveled around t=0.05 s. Despite this difference, the D_{yy} of the temperatures 37 °C and 42 °C are quite similar and the highest among the others. As the temperature decreases, the D_{yy} tends to decrease, reaching a minimum value of about 9×10^{-9} cm^2/s for a temperature of 25 °C. The results from Fig. 6 clearly show a significant difference between D_{yy} at the highest temperatures (37 °C and 42 °C) and D_{yy} calculated at the lowest temperature, i.e., 25 °C. It is widely accepted by the scientific community that the collision frequency of particles (in this particular study RBCs) tends to decrease as temperature reduces (Jia et al., 2007; Nguyen and Wereley, 2006; Thurston and Henderson, 2007). In contrast, when the temperature decreases the plasma viscosity increases (Lim et al., 2010). These well-known phenomena might be the main reasons for the observed D_{yy} decrease by lowering the temperature.

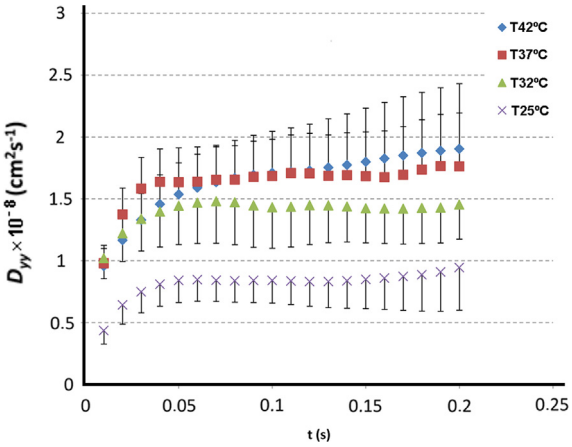

Fig. 6. RBC dispersion coefficient at the middle plane of a 100-μm glass capillary for temperatures ranged from 25 °C to 42 °C. The measured values are expressed as the means ± standard deviation according to a t-test analysis at a 95% confidence interval.

Hence, these findings suggest that the temperature should not be ignored in future RBC D_{yy} studies.

Fig. 7 presents the RBCs radial dispersion coefficient as a function of the temperature where the D_{yy} represents the averaged value of the last three time intervals (t=0.18–0.2 s). The results from Fig. 7 show clearly that the dispersion coefficient increases with the temperature but at 37 °C it tends to level off.

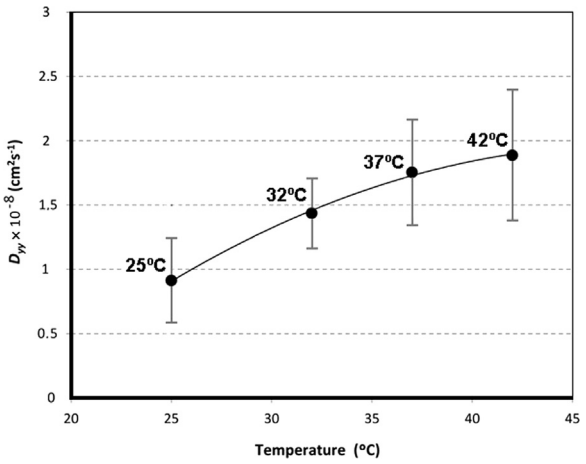

Fig. 7. RBC dispersion coefficient as a function of the temperature where D_{yy} represents the averaged value of the last three time intervals (t=0.18–0.2 s). The measured values are expressed as the means ± standard deviation according to a t-test analysis at a 95% confidence interval.

Blood rheology has been reported to be changed at various physiopathological conditions, such as alterations of hematocrit, RBC deformability, RBC aggregation and temperature. Regarding the temperature, several experimental works have shown remarkable results. Artmann et al. (1998), by using a micropipette technique, have demonstrated that normal RBCs and ghost cells (RBCs without hemoglobin) behave differently by increasing the temperature. When normal RBCs were aspirated at 22 °C into micropipettes, the RBCs tend to block the pipettes, however when the temperature was raised up to 36.4 °C the RBCs start to pass through the micropipettes. For the case of the ghost cells, the transition temperature was 28.3 °C. These findings clearly demonstrate a temperature dependency associated to the RBCs deformability changes. In addition, these findings were attributed to changes of the hemoglobin (major RBC intracellular protein) and spectrin (major RBC membrane protein). However, Mills et al. (2007) by means of optical tweezers have shown that healthy RBC membrane stiffness at 37 °C and 41 °C was similar to healthy RBCs at room temperature. Hence, this work makes us believe that the temperature promotes almost no effect on the RBC membrane stiffness. Recently, experiments performed in microfluidic constriction channels have shown that RBC deformability increases from room temperature to 37 °C (Huang et al., 2010; Quinn et al., 2011). These results were mainly attributed to the reduction of the flow resistance due to an increase in temperature and a consequent reduction of the effective viscosity of the medium (combination of surrounding external fluid, RBC internal fluid and RBC membrane). Our results are in a good agreement with the experimental findings obtained in microfluidic constriction channels and reinforces that there is a strong temperature dependence on the RBC dynamics from room temperature to 37 °C. This temperature dependence was further investigated to evaluate the role of the working fluid in influencing the RBC flow dynamics. Hence, viscosity measurements of our working fluid were performed by means of a stress control rheometer (Malvern/Bohlin CVO). The results have shown that for the temperatures from room temperature (25 °C) to 37 °C the viscosity of our working fluid exhibited a strong temperature dependence, as seen in Fig. 8. At temperatures up to 37 °C the fluid viscosity decreased as the temperature increased. However, at 42 °C the viscosity is similar to the one obtained at 37 °C. This additional experiment reinforces our confocal results showing a strong temperature dependence of the dispersion coefficient at temperatures up to 37 °C. Moreover, Lim et al. (2010) by using a pressure scanning capillary viscometer, have shown that both plasma and whole blood viscosity also decreased as temperature increase (from 4 °C to 37 °C). Overall, our results complement

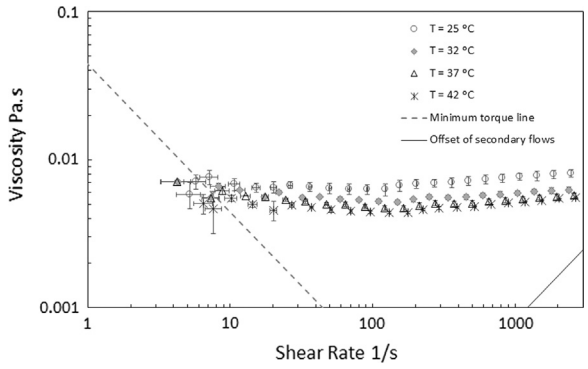

Fig. 8. Steady shear viscosity curves for the working fluid at four different temperatures (25 °C, 32 °C, 37 °C and 42 °C).

previous research findings and suggest that the external fluid surrounding the RBCs plays a major role in reducing the viscosity of the medium and as a result increasing the RBC dispersion coefficient.

3.5. Comparison with other studies

Radial dispersion of flowing RBCs in a capillary is known to be affected by other factors such as the diameter of the capillary and hematocrit of the working fluids (Lima et al., 2008a). Lima et al. (2008a) have examined various hematocrits with 50 μm and 100 μm glass capillaries. The results showed the higher D_{yy} was obtained in the higher Hcts and larger diameter. Saadatmand et al. (2011) also investigated the dispersion of both fluorescent tracer particles and RBCs with several Hcts in the 50 μm glass capillary tube. In this latter study they have observed that an increase of Hct has promoted an increase of the tracer particles D_{yy}. It was also revealed that the dispersion of tracer particles was larger than the observed for RBCs flowing in fluid with 10% Hct. It should be mentioned, that all past experiments were undertaken with a surrounding temperature of about 36–37 °C and Re at 0.001–0.008. Note that Saadatmand et al. (2011) have used a capillary which the diameter was half of the one used in the current study. The first observation from this comparison is that our D_{yy} values are in between the results of Lima et al. (2008a) and Saadatmand et al. (2011). The values obtained by Lima et al. (2008a) were higher than the present results. This small discrepancy might have been due to their use of a higher Hct (15%). In fact the work of Lima et al. (2008a) has showed that D_{yy} tends to increase with the Hct. Additionally, it is possible to notice an appreciable difference between our present results and the values obtained by Saadatmand et al. (2011). We believe that the main reason for the lower values obtained by Saadatmand et al. (2011) is mainly due to the lower diameter used in their study. In fact, Lima et al. (2008a) have demonstrated that D_{yy} tends to decrease with the diameter. This phenomenon is believed to be caused by Hct reduction with the diameter (Fahraeus effect) and also due to the geometric constraints that limits the transverse motion of the RBCs.

4. Conclusions

In this work, we have presented experimental measurements of the effect of temperature on the RBCs dispersion flowing through a 100 μm glass capillary. Labeled RBCs were tracked by means of a confocal micro-PTV system at four different temperatures, i.e., 25 °C, 32 °C, 37 °C and 42 °C (measured temperature ± 1 °C). The results illustrated an enhancement of the cells diffusion as the temperature increases.

Rheological studies, have shown that as the temperature increases, the whole blood and plasma viscosity tends to decrease and

consequently RBCs become less resistant to hydrodynamic dispersion. The present results are in good agreement with these studies, since the radial dispersion coefficient of the RBCs increased almost linearly with the temperature. However, it should be noted that the dispersion coefficient at 37 °C tends to level off. This temperature dependence was further investigated to evaluate the role of the radial positions on the RBCs dispersion coefficient. Results have revealed, that for all the studied temperatures, the highest dispersion was occurred in the region 0.4*R*–0.8*R*. In contrast, the lowest values were obtained at radial positions between 0.8*R* and 1*R*, which corresponds to a cell-depleted region. At this region, we have included in our calculations the rolling RBCs along the microchannel wall, which are likely to be the main cause for the low cell dispersion values obtained at this depleted region. Our findings show clearly a temperature dependence on the RBCs diffusion and as a result the temperature should not be ignored in future *in vitro* blood flow studies. Moreover, these findings provide novel insights regarding the blood transport phenomena in both microchannels and microvessels.

5. Conflict of interest statement

The authors have no conflict of interest to report.

Acknowledgments

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