Dynamic Flow Behaviour of a Blood Analogue Fluid in Microchannels for Microcirculation Studies

I. Gonçalves[1,2], J. Varelas[1], G. Coutinho[1], A. S. Moita[1,3], D. Pinho[2,4,5], R. Lima[2,6], J. M. Miranda[6], E. J. Veja[7], J. M. Montanaro[7] and A. L. N. Moreira[1]

[1]*IN+ - Center for Innovation, Technology and Policy Research, Instituto Superior Técnico, Universidade de Lisboa, Av. Rovisco Pais, 1049-001 Lisboa, Portugal*

[2]*Metrics, Mechanical Engineering Department, University of Minho, Campus de Azurém, 4800-058, Guimarães, Portugal*

[3]*CINAMIL – Military Academy Research Center, Department of Exact Sciences and Engineering, Portuguese Military Academy, R. Gomes Freire, 203, 1169-203 Lisbon, Portugal*

[4]*Center for MicroElectromechanical Systems (CMEMS - UMinho), University of Minho, Campus de Azurém, Guimarães, 4800-058 Portugal*

[5]*INL, International Iberian Nanotechnology Laboratory, Av. Mestre José Veiga, 4715-330 Braga, Portugal*

[6]*CEFT, Faculdade de Engenharia da Universidade do Porto (FEUP), R. Dr. Roberto Frias, 4200-465 Porto, Portugal*

[7]*Dept. de Ingeniería Mecánica, Energética y de los Materiales and Instituto de Computación Científica Avanzada (ICCAEx), Universidad de Extremadura, 06006 Badajoz, Spain*

Keywords: Blood Analogue Fluid, Fluid Characterization, Surfactant Concentration, Microcirculation, Microfluidics.

Abstract: This study proposes a simple, stable and low cost 2-phase blood analogue fluid, which can mimic multiphase phenomena of real flow in microcirculation. This analogue fluid is mainly composed of Brij L4 surfactant suspended in pure water. The analogue fluid is compared with real blood, both in terms of thermophysical properties as well as in terms of its dynamic fluid flow behaviour, for different concentrations of the surfactant. The results on the particle size distribution confirm the reproducibility of the fluid preparation, as well as of its stability. The analogue fluid density is close to that of water, thus approaching the blood density. As for the rheology, the blood analogue fluid depicts a shear thinning behaviour, matching that of blood, except for very high Brij L4 concentrations. Fluid flow experiments show that the blood analogue can generate cell-free layers (CFL), with thickness close to that of real blood, which corroborates that the proposed analogue is able to mimic blood flow phenomena in microvessels. Increasing the surfactant concentration promotes the augmentation of the CFL's, but also endorses agglomeration and clogging. Flow separation occurs also at the highest surfactant concentrations, which makes more difficult for the particles to follow the flow, so that flow field evaluation becomes more problematic.

1 INTRODUCTION

Blood flow phenomena in microcirculation has been studied both in vivo (Tateishi *et al.*, 1994; Kim *et al.*, 2006; Namgung *et al.*, 2014) and in vitro (Abkarian *et al.*, 2008; Tripathi *et al.*, 2015; Bento *et al.*, 2018; Catarino *et al*., 2019). Despite of the significant advances in this field, reported in the last decade, understanding of blood flow phenomena at both physiological and pathological conditions is still not yet completely understood nor described.

In biomicrofluidics experiments, it is a common practice to use in vitro blood to investigate blood flow phenomena observed in real microvessels, such as the plasma layer or cell-free layer (CFL) and the bifurcation law effect (Completo *et al.*, 2014, Pinho *et al.*, 2017; Catarino *et al*., 2019). However, handling real blood fluids is not straightforward due to several difficulties such as sanitary, bureaucratic and technical problems (Sousa *et al.*, 2011; Campo-Deano *et al.*, 2013). These issues have constrained the use of blood in long term flow experiments. Hence, it

Gonçalves, I., Varelas, J., Coutinho, G., Moita, A., Pinho, D., Lima, R., Miranda, J., Veja, E., Montanaro, J. and Moreira, A.
Dynamic Flow Behaviour of a Blood Analogue Fluid in Microchannels for Microcirculation Studies.
DOI: 10.5220/0010343901750181
In *Proceedings of the 14th International Joint Conference on Biomedical Engineering Systems and Technologies (BIOSTEC 2021) - Volume 1: BIODEVICES*, pages 175-181
ISBN: 978-989-758-490-9

is crucial to develop a simple and stable blood analogue with flow properties close to real blood.

One of the first blood analogue fluids, used to perform flow experiments, were Newtonian fluids composed of mixtures of water and glycerol (Nguyen *et al.*, 2004; Yousif *et al.*, 2011; Deplano *et al.*, 2014). Later, these fluids were improved to mimic the non-Newtonian behaviour of human blood (Sousa *et al.*, 2011; Campo-Deano *et al.*, 2013). However, all those were homogeneous and were not able to mimic multiphase phenomena of real blood in microcirculation. Recently, several research studies report the development of blood analogue fluids containing solid suspended microparticles, which can mimic the multiphase effects of blood (Calejo *et al.*, 2016; Pinho *et al.*, 2017, 2019). Nevertheless, these analogues have major drawbacks such as strong aggregation tendency and consequent blockage of the microchannels. Thus, it is important to develop aggregation-free particulate blood analogues and test them in representative experiments.

In this context, and following our previous work (Moita *et al.*, 2019), the present study proposes a simple, stable particulate blood analogue, which can mimic multiphase phenomena of real blood in microcirculation. The proposed fluid is composed of Brij L4 surfactant suspended in pure water. The analogue fluid is characterized and compared with real blood. Early results addressed deformability behaviour in microchannels with a sudden contraction (Moita *et al.*, 2019) as well as the effect of the constriction to generate cell-free layer at the constriction downstream (Lima *et al.,* 2020). In this work, additional information is provided on the effect of the surfactant in the fluid viscosity and surface tension and the behaviour of the biomimetic fluid in a bifurcation.

2 MATERIALS AND METHODS

2.1 Preparation and Characterization of the Analogue Fluid

The surfactant Brij L4 has tendency to form stable spherical clusters. Hence, in this work, 0.5wt% to 10wt% of Brij L4 surfactant was used to produce the proposed blood analogue fluid. Briefly, the surfactant was mixed with pure water. After the accomplishment of a homogeneous mixture, the fluid was forced to flow through precolumn filters having a membrane with an average pore size of 20 μm. This precolumn filter allows the generation of smaller and more homogenized surfactant droplets.

The biomimetic fluid was characterized in terms of density ρ, surface tension σ_{lv} and viscosity μ. Furthermore, the wettability of the prepared solutions with Polydimethylsiloxan PDMS (the material of the microchannels), was also evaluated based on the static contact angle.

Density was evaluated using a picnometer. The measured value, 996 kgm^{-3} is close to that of water, as expected. The viscosity was measured using a rheometer (Bohlin CVO, Malvern, Worcestershire, UK) using a coneplate geometry, with a diameter of 55 mm and an angle of 1° with a gap size of 0.03 mm.

The steady shear viscosity curves were obtained over a wide range of shear rates, ranging between 10 s^{-1} and 10000 s^{-1}.

Surface tension was measured on an optical tensiometer (THETA, from Attention), using the pendant droplet method. The final surface tension value evaluated for each solution was averaged from 15 measurements. All the measurements depict standard mean errors lower than 0.35.

Finally, the wettability of the solutions with the material that was used to fabricate the microchannels – PDMS, was quantified with the static contact angle θ_e, measured with the optical tensiometer THETA, from Attention, using the sessile drop method. Images with a resolution of 640×480 pixels are post-processed by a drop detection algorithm based on Young-Laplace equation (One Attention software). The accuracy of these algorithms is argued to be of the order of ± 0.1º (Cheng, 2008). For the current optical configuration, the spatial resolution is 15.6 μm/pixel.

Detailed description of the techniques used in the characterization of the thermophysical properties of the fluid can be found in Pereira *et al.* (2014) and in Moita *et al.* (2016, 2018).

2.2 Characterization of the Particles Size Distribution using Laser Confocal Fluorescence Microscopy

The size distribution of the particles suspended in the solutions was evaluated using a Laser Scanning Confocal Microscope (SP8 from Leica). The images were obtained with a 10X objective lens and recorded with a resolution of 512×512 pixels. Rhodamine B (Sigma Aldrich) was added to the solutions, with a concentration of $3.968x10^{-6}$ g/mL, which does not alter the thermophysical properties of the analogue fluid (Moita *et al.*, 2019). For this dye, an excitation laser with a wavelength of 552 nm was used, fixing the laser power to 10.50 mW (3% of its maximum power). The gain of the microscope photomultiplier

was fixed at 550 V. These values were chosen after a sensitivity analysis on the contrast of the image (before the post-processing) and on the Signal to Noise Ratio (SNR). An in-house code, developed in MATLAB was then used to process the 1024×1024 pixels images, which were taken with a scanning frequency of 400 Hz.

2.3 Characterization of the Analogue Fluid Flow in the Microchannels

To study the dynamic behaviour of the analogue fluid flow and its ability to generate the CFL's, flow experiments were performed in two microchannels, one with an abrupt contraction and one with a bifurcation. The microchannels were fabricated in PDMS using soft lithography, following the fabrication method described in Faustino *et al.* (2016). The depth of the microfluidic device was around 30 µm. Figure 1 shows a schematic drawing of the microfluidic devices used in this study, including their main dimensions. Table 1 depicts the main dimensions of the bifurcated channel represented in Figure 1b.

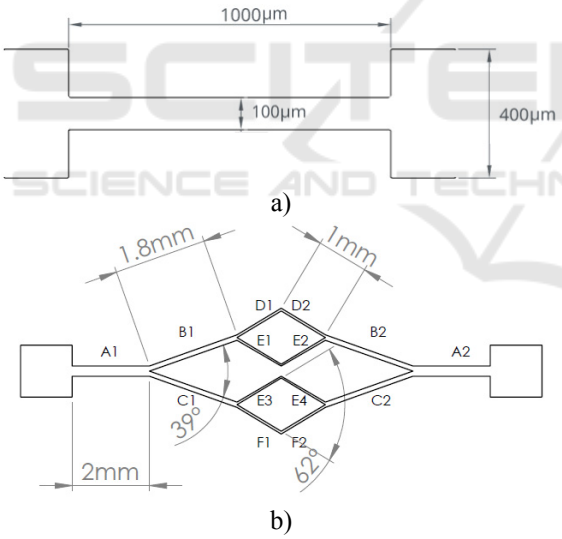


Figure 1: Schematic with the geometry and main dimensions of the microchannel used to study the analogue fluid flow behaviour and CFL's generation. a) microchannel with an abrupt constriction, b) microchannel with a bifurcation.

The flow was driven at constant pressure using a syringe pump (KD Scientific, USA). Images were taken using a high-speed camera (Phantom v7.1; Vision Research, USA), with a resolution of 640×640 pixels and with a frame rate of 4000 fps. The lens used had a 20X magnification and the spatial resolution for this optical configuration was 1.185 pixel/µm. The images were processed using the software ImageJ (1.46r, NIH, USA). Regarding, the CFL measurements, the recorded image sequences were evaluated using the function "Z project" from the ImageJ software.

Table 1: Main dimensions of the bifurcated microchannel represented in Figure 1b). All the microchannels have a height of 50mm.

Section	Width (µm)
$A_1=A_2$	200
$B_1=B_2$	118.1
$C_1=C_2$	84.8
$D_1=D_2$	58.19
$E_1=E_2=E_3=E_4$	46.55
$F_1=F_2$	23.29

3 RESULTS AND DISCUSSION

3.1 Particle Size Distribution of the Analogue Fluid

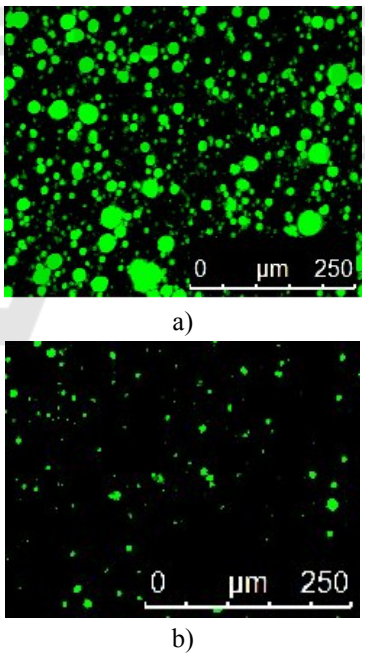


Figure 2: Images of the analogue fluid prepared with the surfactant, obtained by Laser Scanning Fluorescent Confocal Microscopy (objective of 20x magnification and 0.75x numerical aperture) after filtering the solution with a 20 µm precolumn filter. a) without filtering, b) after passing the filter.

As reported in Moita *et al.* (2019), the initial scope for the development of this analogue fluid was to mimic biological fluids. Hence, images taken at the earliest stages of this research with the confocal microscope, as reported in Moita *et al.* (2019) show that the devised solutions present an heterogeneous precipitated of deformable particles, depicting a wide range of particles sizes (between 2.5 mm and 40 mm). Deformability experiments however, showed a very good ability of these particles to mimic red blood cells. To make the fluid more homogeneous in terms of particles number and size distribution, Lima *et al.* (2020) propose the use of a precolumn filter. Following such procedure, the fluid becomes more homogeneous, as shown in the images taken with the Laser Scanning Confocal Microscope, depicted in Figure 2.

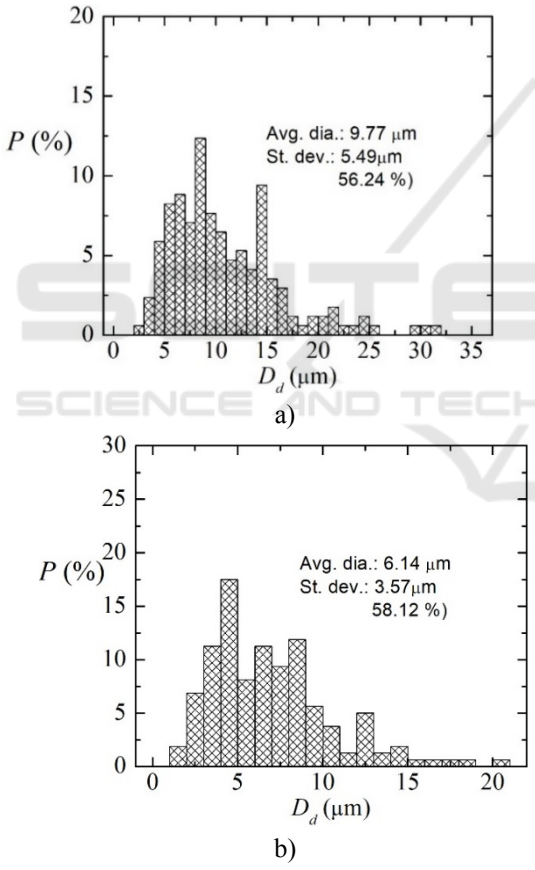


Figure 3: Probability distribution for the surfactant droplet diameter (Dd) generated a) without filtering, b) after passing the filter.

The homogeneity of the fluid after filtering is quantitatively confirmed by the particles size distribution obtained by image processing, as shown in Figure 3. The figure also shows that the average diameter of the surfactant droplets was reduced from 9.77 μm to 6.14 μm (size closer to that of red blood cells) because of the precolumn filter. Additionally, whereas the unfiltered fluid presented droplets larger than 30 μm, a post-filtering analysis revealed a maximum diameter of approximately 20 μm. The standard deviation (presented both in absolute values and in percentage) is also mildly reduced. Hence, summing up, results show that the precolumn filter used in this study enables the generation of smaller and less polydisperse surfactant microdroplets.

3.2 Effect of the Surfactant Concentration in the Thermophysical Properties of the Analogue Fluid

After controlling the size and distribution of the particles on the analogue fluid, it is relevant to infer on the adequate concentration of surfactant to use to match the properties of the analogue fluid with those of blood. In this context, this work addressed the use of different Brij L4 concentrations, ranging between 0.5wt% and 2wt%. Being water-based solutions, their density, evaluated with a pycnometer, was always close to that of water (approximately 996 kgm^{-3}), regardless of the concentration of Brij L4. This is an expected result given the high density of water, when compared to that of the surfactant and given the still relatively low mass concentrations of surfactant used.

Table 2: Effect of the concentration of Brij L4 in the surface tension of the resulting analogue fluids and in the static contact angle obtained with a PDMS surface.

Brij L4		Surface tension σ_{lv} (mNm^{-1})	Static contact angle θ_e (º) [PDMS]
Concentration	Filter (μm)		
Water	-	72.90	68.92
Brij L4 0.5wt%	20	27.21	65.47
Brij L4 5wt 1%	10	31.74	60.29
	20	31.61	59.69
	10+20	31.62	59.00
Brij L4 5wt 2%	10	31.74	58.45
	20	31.74	51.00
	10+20	31.69	48.66
Brij L4 5wt 5%	10	31.80	47.85
	20	31.87	47.28
	10+20	31.82	42.05
Brij L4 5wt 10%	20	26.20	59.81

A different trend is nevertheless observed for the surface tension. Hence, given that the surfactants are

usually used to alter the surface tension of other liquids, the surface tension of the Brij L4 solutions is significantly reduced, when the surfactant is added, even for the lowest concentration of Brij L4 (0.5wt%). However, following this decrease in the surface tension value, observed for the analogue fluid with the lowest surfactant concentration, when compared to water, a stable plateau value is then obtained, as the concentration of surfactant is increased, up to the maximum value tested here.

Consistently, the contact angle measured for the analogue solutions was also kept within a constant value, for increasing concentration values. Such trends can be observed in Table 2.

Finally, it is vital to infer on the effect of adding the surfactant to the rheology of the resulting analogue fluid. Rheology curves obtained at 1% were compared with those measured at 5% and at 10% (in weight), as reported in Lima *et al.* (2020) as well as with the viscosity curve for human blood (Lima *et al.*, 2020). These curves are depicted in Figure 4.

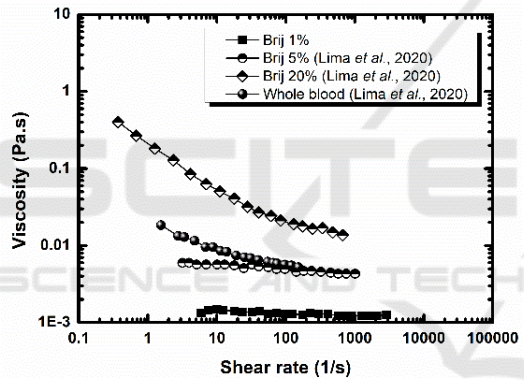


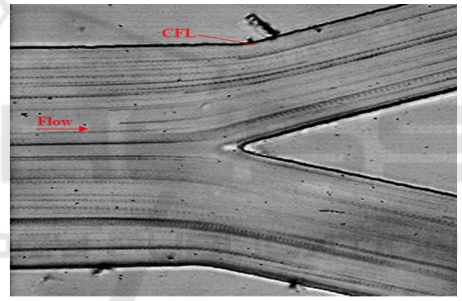
Figure 4: Steady shear viscosity curves for the analogue fluid with a concentration of 1%, 5% and 10% of Brij L4, and human whole blood.

Figure 4 depicts an increase in the analogue fluid viscosity as one increments the concentration of Brij L4. However, while the viscosity increases almost linearly with the shear rates for low surfactant concentrations (up to 5wt%), a strong shear thinning behaviour is observed for the analogues containing very high Brij L4 concentrations (larger than 10wt%). Despite of the known shear-thinning behaviour of blood (endorsed by the behaviour of the red blood cells), the viscosity curves of the analogue fluids with higher surfactant concentrations do not match the curve of the whole blood, which is closer to that of the analogue fluids with lower surfactant concentrations (lower than 5%).

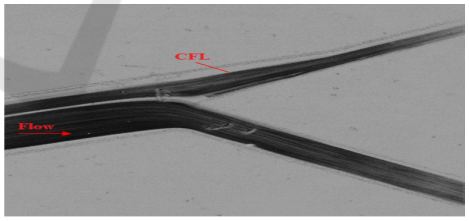
Given this trend, only these concentrations were considered in the characterization of the flow behaviour of the analogue fluid, as discussed in the following sub-section.

3.3 Fluid Flow of the Analogue Fluid in Microchannels

The tendency of the red blood cells to migrate to the center of the microchannels or microvessels originates the formation of a cell depleted layer around the walls, known as the cell-free layer (CFL). This is a well-known phenomenon that occurs in microfluidic devices and microvessels with dimensions lower than 300 µm. Hence, in this study flow visualizations allowed observation of the CFL thickness for the proposed blood analogue and *in vitro* blood flowing through a microchannel with an abrupt contraction. Figure 5 shows treated images for the tested fluids for a flow rate of 15 µL/min. The results show that, at the downstream region of the microchannel contraction, there is a high propensity for CFL formation both for the analogue fluid and for blood.



a)

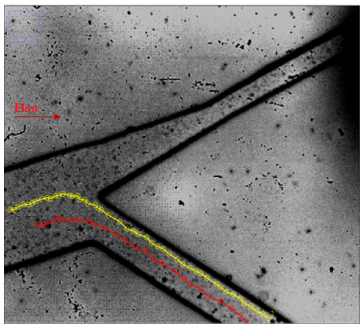


b)

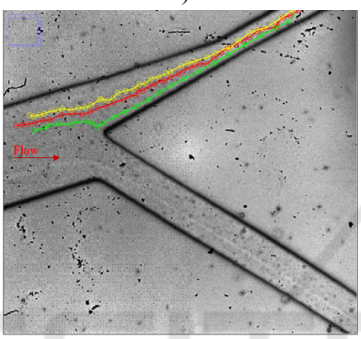
Figure 5: Flow and CFL visualization of: a) proposed blood analogue, b) in vitro blood.

In addition, the measurements of the CFL thickness for both fluids are in good agreement which indicates that the proposed blood analogue fluid is able to mimic blood flow phenomena happening in microvessels and in microfluidic devices, such as CFL and cross flow filtration. The CFL is also generated at the bifurcated microchannel, as observed in Figure 6. Analogue fluids with higher surfactant concentration tend to generate a thicker CFL.

However, aggregation and clogging occur more often, and separation is also easier to occur, given the higher viscosity which decelerates the fluid and dissipates the momentum in the boundary layer.



a)



b)

Figure 6: Flow and CFL visualization for the analogue fluid, for different concentrations of Brij L4. a) and CFL measurements of the a) 1% Brij L4, b) 2% Brij L4 (in weight).

Analysing the particles velocity, and considering the average velocity, as shown in Table 3, one can observe an acceleration of the particles after the bifurcation, which can be related to mass conservation principles: as the cross section of the bifurcation channels is smaller than that of the main channel, the flow will accelerate, by mass flow conservation. These results were obtained for the 1% Brij L4 solution and for a flow rate of 1ml/min, but similar trends were inferred for other surfactant concentrations/flow rates, apart from the aforementioned differences. The results also show that the velocity of the particles near the center of the channel (the red trajectory in Figure 6a) have higher velocities than those closer to the wall. This means that the particles are following the flow, which has characteristically its maximum velocity at the center of the channel, while the particles more apart from the center are more affected by the wall effects.

Table 3: Characterization of the velocity of the particles identified in the red and yellow trajectories in Figure 6. The flow is the analogue solution with 1% Brij L4 (in weight) after passing the 20 mm filter. The flow rate used is 1mL/min.

Location	Average particle velocity (μm/s)
Particle 1 (red trajectory in Figure 6) – before bifurcation	3.14
Particle 1 (red trajectory in Figure 6) – after bifurcation	5.08
Average vel. particle 1	4.39
Particle 2 (yellow trajectory in Figure 6) – before bifurcation	2.23
Particle 2 (yellow trajectory in Figure 6) –after bifurcation	3.36
Average vel. particle 2	2.90

4 CONCLUSIONS

The present work proposes a low-cost and stable blood analogue fluid, for microcirculation studies. This analogue fluid is based on water solutions with surfactant Brij L4. Following our previous work, this study infers on the effect of the surfactant concentration in the particle distribution and in the thermophysical properties of the resulting fluid. Furthermore, the dynamic flow and CFL generation are also evaluated. The thermophysical properties of the analogue fluid are observed to be close to those of real blood. This includes the shear thinning behaviour, which is only deviated for very high concentrations (>10%). Larger surfactant concentrations (of the order of 1-2% promote the generation of the CFL, but also endorses agglomeration and clogging.

ACKNOWLEDGEMENTS

This work was supported by Fundação para a Ciência e a Tecnologia (FCT) under the context grants UIDB/04077/2020, UIDB/04436/2020 and UIDB/00532/2020 and of project JICAM/0003/2017, in Projecto 3599 - Promover a Produção Científica, o Desenvolvimento Tecnológico. Authors also acknowledge FCT for supporting I. Gonçalves with a research fellowship through project LISBOA-01-0145-FEDER-030171/ PTDC/EME-SIS/30171/2017.

REFERENCES

Abkarian, M., Faivre, M., Horton, R., Smistrup, K., Best-Popescu, C. A., Stone, H. A., 2008. Cellular-scale hydrodynamics, *Biomedical Materials*, 3:034011.

Calejo, J.; Pinho, D.; Galindo-Rosales, F.J.; Lima, R.; Campo-Deaño, L., 2016. Particulate Blood Analogues Reproducing the Erythrocytes Cell-Free Layer in a Microfluidic Device Containing a Hyperbolic Contraction. *Micromachines*, 7:4.

Campo-Deaño, L., Dullens, R. P. A., Aarts, D. G. A. L., Pinho, F.T., Oliveira, M. S. N., 2013. Viscoelasticity of blood and viscoelastic blood analogues for use in polydymethylsiloxane in vitro models of the circulatory system. *Biomicrofluidics*, 7:034102.

Catarino, S. O., Rodrigues, R. O., Pinho, D., Miranda, J. M., Minas, G., Lima, R., 2019. Blood Cells Separation and Sorting Techniques of Passive Microfluidic Devices: From Fabrication to Applications. *Micromachines*. 10:593.

Cheng, P., 2008. Automation of Axisymmetric Drop Shape Analysis using Digital Imaging Processing, *PhD thesis, University of Toronto, Canada*.

Completo, C., Geraldes, V., Semiao, V., 2014. Rheological and dynamical characterization of blood analogue flows in a slit. *Int. J. Heat Fluid Flow*. 46:17-28.

Deplano, V., Knapp, Y., Bailly, L., Bertrand, E., 2014. Flow of a blood analogue fluid in a compliant abdominal aortic aneurysm model: Experimental modelling. *J. Biomech*, 47:1262–1269.

Faustino, V., Catarino, S. O., Lima, R., Minas, G., 2016. Biomedical microfluidic devices by using low-cost fabrication techniques: A review, Journal of *Biomechanics*, 49 (11):2280–2292.

Kim, S., Kong, R. L., Popel, A. S., Intaglietta, M., Johnson, P. C., 2006. A computer-based method for determination of the cell-free layer width in microcirculation. *Microcirculation* 13(3):199-207.

Lima, R., Vega, E. J., Moita, A. S., Miranda, J. M., Pinho, D., Moreira, A.L.N., 2020. Fast, flexible and low-cost multiphase blood analogue for biomedical and energy applications. *Exp. Fluids*, 61:231 (11 pages).

Moita, A. S., Laurência, C., Ramos, J.A., Prazeres, D. M. F., Moreira, A. L. N., 2016. Dynamics of droplets of biological fluids on smooth superhydrophobic surfaces under electrostatic actuation, *J. Bionic Eng.*, 13:220-234.Moita, A.S., Caldeira, C., Golçalves, I., Lima, R., Veja, E. J., Moreira, A. L. N., 2020. Analogue fluids for cell deformability studies in microfluidic devices. Biomedical Engineering Systems and Technologies. Series: Communications in Computer and Information Science, Springer International Publishing AG, part of Springer Nature Switzerland, A. Roque et al. (Eds.): BIOSTEC 2019, CCIS 1211, pp. 1–12, 2020.

Namgung, B, Liang, L. H., Kim, S., 2014. Physiological significance of cell-free layer and experimental determination of its width in microcirculatory vessels. In: *Lima et al. editors. Visualization and simulation of complex flows in biomedical engineering. Dordrecht: Springer*; p. 75–87.

Nguyen, T.T., Biadillah, Y., Mongrain, R., Brunette, J., Tardif, J.-C., Bertrand, O.F., 2004. A Method for Matching the Refractive Index and Kinematic Viscosity of a Blood Analog for Flow Visualization in Hydraulic Cardiovascular Models. J. Biomech. Eng., 126, 529–535.

Pereira P., Moita, A. S., Monteiro, G., Prazeres, D. M. F., 2014. Characterization of English weed leaves and biomimetic replicas. Journal of Bionic Engineering, 11(3):346-359.

Pinho, D., Campo-Deaño, L., Lima, R., Pinho, F.T., 2017. In vitro particulate analogue fluids for experimental studies of rheological and hemorheological behavior of glucose-rich RBC suspensions. *Biomicrofluidics*, 11:054105.

Pinho, D., Muñoz-Sánchez, B. N., Anes, C. F., Vega, E. J., Lima, R., 2019. Flexible PDMS microparticles to mimic RBCs in blood particulate analogue fluids. *Mechanics Research Communications*. 100:103399.

Sousa, P.C., Pinho, F.T., Oliveira, M. S. N., Alves, M. A., 2011. Extensional flow of blood analogue solutions in microfluidic devices. *Biomicrofluidics*, 5:14108.

Tateishi, N, Suzuki, Y, Soutani, M, Maeda, N., 1994. Flow dynamics of erythrocytes in microvessels of isolated rabbit mesentery: cell-free layer and flow resistance. *J. Biomech.* 27:1119–1125.

Tripathi, S, Bala, Varun, Kumar, Y. V., Prabhakar, A., Joshi, S. S., Agrawal, A., 2015. Passive blood plasma separation at the microscale: a review of design principles and microdevices. *J Micromech Microeng.* 25:8.

Yousif, M. Y., Holdsworth, D. W., Poepping, T. L., 2011. A blood-mimicking fluid for particle image velocimetry with silicone vascular models. *Exp. Fluids*, 50:769–774.