Simulation and experimental evidence for the decrease of platelet margination with an increase in volume fraction of stiffened red blood cells in flow

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1. Introduction

Whole blood is a suspension of cells, red blood cells (RBCs), platelets, and white blood cells, in a protein rich plasma that collectively has a non-Newtonian rheology. RBCs are the most numerous blood cells and due to their deformability and bi-concave shape the RBC contributes significantly to the complex rheology of whole blood. Pathologies have been found to affect the deformability of the red blood cell such as Diabetes, Sickle Cell Anemia [1], and HIV. In this research we perform numerical and experimental analysis on the effects and outcomes of the presence of stiffened RBCs on haematocrit profiles and platelet margination in flowing whole blood.

2. Methods

We perform cell resolved blood flow simulations in both two and three dimensions using the dense cellular suspension codes HemoCell2D [2] and HemoCell [3]. Both codes use the lattice Boltzmann method to model the blood plasma and a discrete element method for the material model of both red blood cells (RBC) and platelets, the material model is coupled to the fluid via the immersed boundary method. In two dimensions we perform whole blood simulations of 40% haematocrit in a straight periodic channel with a width of 127μm with varying stiffened RBC fractions of 0 to 100% and varying wall shear rates (WSR) of 200, 500 and 1000s^{-1}. The two dimensional simulations provide a computationally inexpensive parameter sweep to understand what to study in more detail in three dimensions. In three dimensions we provide a stiffened RBC model validated on the ektacytometry measurements [4] of stiffened RBCs using tert-Butyl hydroperoxide (TBHP) across a range of shear rates. We simulate 30% of flowing whole blood in a periodic pipe flow geometry with stiffened RBC fractions of 0, 50, and 100% at a WSR of 1000s^{-1}.

We measured, via confocal microscopy, platelet distributions by flowing 30% haematocrit whole blood through a 100μm wide glass channel with varying stiff RBC volume fractions of 0, 30, 50, 70 and 100% at WSRs of 500 and 1000s^{-1}. Platelets are fluorescently tagged with Allophycocyanin (APC) anti-human CD41/CD61 and their populations are measured at 2μm intervals from the bottom to the top of the channel by taking the mean image intensity at each height. Each z-stack image is 150x150μm in

spatial resolution. Due to the high haematocrit of 30% the attenuation of light through the flowing blood limits the depth at which we can reliably measure the tagged platelets. We performed uv-vis spectrophotometry to quantify the amount of light loss, by directly measuring the absorbance, from the platelets given a range of haematocrits of 5, 10, 20, 30 and 40% and a range of stiffened RBC fractions of 0, 30, 50, 70, and 100%. We report that more than 90% of the emitted platelet's signal is transmitted at a distance of 4μm. For this reason we measure and report the platelet concentration located distance of 4μm from the channel wall.

3. Results

We report in our two dimensional simulations of a 127 μm wide channel a decrease in platelet concentration at 5μm from the wall as the stiff RBC volume fraction increases. This effect is present for the all of the WSR cases and is highlighted in Figure 1.

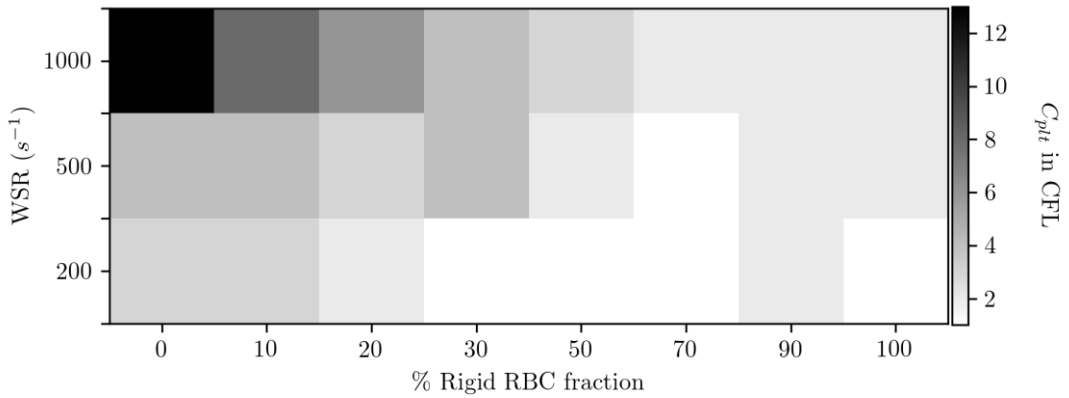

Figure 1 *Platelet concentration at 5* μm *from the vessel wall as a function wall shear rate and stiff volume fraction in a two dimensional cell resolved blood flow simulation.* C_{plt} *is the platelet concentration, normalized to the mean channel volume fraction.*

In the three dimension simulations we also observe the decrease of platelet concentration at the wall as stiff RBC volume fraction increases. We observe both in 2D and 3D the decrease of the red blood cell free layer. In a 50:50 mixture of stiff:healthy RBCs we report that healthy RBCs dominate a region close to but not at the wall, approximately at a radius range of 28μm < R < 45μm.

With confocal microscopy we measure the decrease of platelet signal at a distance of 4μm from the channel wall. This effect is evident in both WSR cases of 500 and 1000s^{-1}. The decrease of platelet concentration at the channel wall is shown in Figure 2.

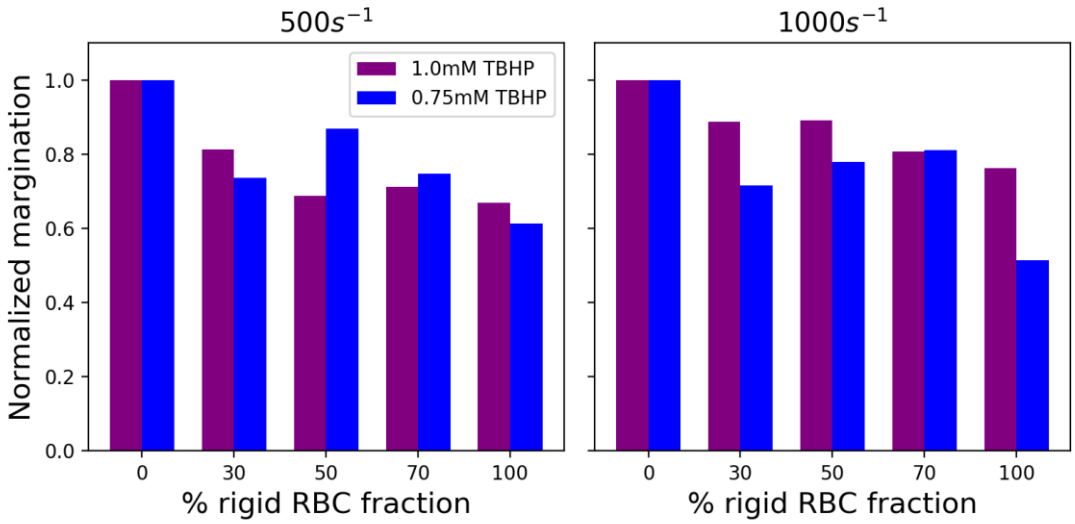

Figure 2 *Normalized margination signal as a function of stiff RBC volume fraction in a 100μm channel. The normalized margination is defined as the image intensity of fluorescently tagged platelets at a distance of 4μm from the wall divided by the platelet image intensity of the 100% healthy RBC case at 4μm from the wall. The stiffened RBCs with 1.0mM THBP are shown in purple, and the 0.75mM THBP are shown in blue.*

Here platelet concentration at the wall is reported as normalized margination which is the average image intensity of platelets at each stiff RBC volume fraction divided by the image intensity of platelets in the 100% healthy RBC case. Results of the measurement shown here are average over three different donors each with three separate measurements at 4μm at each WSR. The platelet signal reported in Figure 2 is normalized with the absorbance per micron of 30% haematocrit of whole blood, as measured via uv-vis spectrophotometry for each stiff:healthy RBC mixture. We anticipate that this effect is heavily dependent on each separate donor and is the reason why we do not see an incremental decrease of margination as we increase the stiff RBC fraction. We do however see a decrease in platelet concentration at the wall generally as stiffened RBCs are present.

4. Conclusions

We observe a decrease in platelet concentration at the vessel wall, i.e. margination, as the volume fraction of stiffened RBCs increases. This is observed first in numerical simulations of cell resolved blood flow in 2D and confirmed in greater detail in 3D. Finally this decrease in platelet concentration at the wall is observed experimentally via confocal microscopy using fluorescently tagged platelets. We believe that the primary factor for this decrease the lift force felt by the RBCs close to the channel wall. Stiffened RBCs have a less net lift force an therefore cause a lessened red blood cell free layer, which allows less platelets to be trapped in this region.

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