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Red blood cell aggregate flux in a bifurcating microchannel

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ABSTRACT

Red blood cell aggregation plays a key role in microcirculatory flows, however, little is known about the transport characteristics of red blood cell aggregates in branching geometries. This work reports on the fluxes of red blood cell aggregates of various sizes in a T-shaped microchannel, aiming to clarify the effects of different flow conditions in the outlet branches of the channel. Image analysis techniques, were utilised, and moderately aggregating human red blood cell suspensions were tested in symmetric (~50–50%) and asymmetric flow splits through the two outlet (daughter) branches. The results revealed that the flux decreases with aggregate size in the inlet (parent) and daughter branches, mainly due to the fact that the number of larger structures is significantly smaller than that of smaller structures. However, when the flux in the daughter branches is examined relative to the aggregate size flux in the parent branch an increase with aggregate size is observed for a range of asymmetric flow splits. This increase is attributed to size distribution and local concentration changes in the daughter branches. The results show that the flow of larger aggregates is not suppressed downstream of a bifurcation, and that blood flow is maintained, for physiological levels of red blood cell aggregation.

1. Introduction

Microfluidic blood flows have received considerable attention in recent years due to the opportunities these systems offer for research on fundamental phenomena occurring at the microscale, and for the development of lab on chip and diagnostic systems. Studying blood flow at such scales allows key flow and structural characteristics of blood, arising from its multiphase nature, to be studied in great depth [1,2]. The complex characteristics of microscale blood flow are mainly due to the presence of red blood cells (RBCs) which constitute the majority of the cellular matter of blood. The red blood cell is a deformable corpuscle resembling a disk (~8 μm diameter) with its centre compressed (thicknesses at the edge and centre are ~2.8 and ~1.4 μm respectively) and accounts for ~45% of the volume of the fluid. The concentration of RBCs in the plasma, which is the continuous phase of the fluid, and their deformability play a part in rendering blood a non-Newtonian fluid; however, it is the tendency of RBCs to aggregate that mainly causes the distinctive increase of blood viscosity at low shear rates. Like most suspensions, blood exhibits a shear thinning behaviour, which is due to the reversible RBC aggregation phenomenon at low shear rates. RBC aggregation occurs mainly in the presence of the plasma protein fibrinogen [3] and is diminished by shear forces developed in the flow. The viscoelastic properties of blood have been examined in early [4–6] as well as more recent studies [7,8], illustrating the weakly-attractive suspension nature of blood. The fact that the flow characteristics may play a role on the aggregation of RBCs has been observed in the studies of Tomaiuolo et al. [9] and Claveria et al. [10], where RBC clustering in microconfined Poiseuille flow is observed independent of aggregative forces. The cluster length was observed to be pressure drop dependent and the formation of larger clusters was favoured by longer residence times in the shear conditions tested [9]. In the study of Clavería et al. [10], red blood cell suspensions in physiological buffer solutions (PBS) and in Dextran solutions at different concentrations were used to mimic healthy and pathological levels of fibrinogen in capillary configurations. It was found that there is a strong increase in the number of isolated cells between the low and the high stress flow cases implying more intense aggregation in the low stress cases. In the same study it was found that at smaller velocities, the cells are mainly in the axial-centred parachute configuration, but at higher velocities RBCs are found in an off-centred position.

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Our previous work on red blood cell transport in straight and bifurcating microchannels (Sherwood et al. [11–13] and Kaliviotis et al. [14,15]) has investigated the cell depleted layer (CDL) formed near the walls, the spatial variation of RBC concentrations, the phenomenon of plasma skimming and the aggregate size distribution. A comprehensive literature review on these characteristics for *in vivo* and *in vitro* studies was also provided therein.

The aggregate size distribution in the flow was examined by the authors for flow in a straight rectangular microchannel (250×50 μm width and height, respectively) through bulk and local aggregation indices [14]. A local aggregation index, A*, based on detecting the iso-intensity patterns formed by the aggregated cells was developed to characterise the aggregate size. This index allowed the quantification of the organisation of aggregates in the plane of shear and highlighted the combined effect of haematocrit and flow velocity on local aggregation characteristics. In the aforementioned study it was also shown that large RBC aggregates show a preference towards the central locations of the channel and this was explained in view of the lower shear forces developed in the regions, compared to those developed close to the boundaries of the flow configuration. The same methodology was recently applied to RBC flows in a T-type bifurcating microchannel [15] in order to examine the aggregate size characteristics. The experiments were performed with aggregating and non-aggregating samples and the effect of the daughter branch flow rate ratio (i.e. the ratio of daughter branch flow rate to this in the parent branch) on the aggregate size characteristics was examined. The results showed that the mean aggregate size decreases in the lower flow rate branches and this was attributed to two characteristics: (a) the existence of regions near the channel walls in the parent branch, which are depleted by aggregates of certain sizes, and (b) to the change in the exact flow split location in the outlet of the parent branch with the change in the flow ratio.

RBC aggregate size distributions have also been considered in the studies of Mehri et al. [1] and Yeom et al. [2]. Yeom et al., used a technique based on counting the number of RBCs per aggregate at low haematocrits (10%), in a very thin chamber (10 μm height) while Mehri et al. quantified the distribution of RBC aggregates in a rectangular geometry (110×60 μm width and height) and at low haematocrits (5, 10 and 15%) using image processing techniques. It was shown that the average aggregate size in the microchannel decreased with shear rate. For example, for a 15% haematocrit the aggregate size decreased from $\sim$160 μm^2 to $\sim$110 μm^2 for pseudo-shear rates of 2.5 and 7.4 s^{-1}, respectively. In the 2.5 s^{-1} case, 41% of aggregates contained 6 or more RBCs, compared to only 16% for the 7.4 s^{-1} case.

The aforementioned studies used image analysis techniques for the characterisation of aggregate size, as they provide unequivocal data [16]. However, there are certain limitations in the use of image analysis for aggregate characterisation, with the main one been the RBC concentration, which affects the image quality due to high light absorption. Kaliviotis et al. [14] addressed these imitations by developing advanced image processing techniques, taking advantage of the specific features of RBC aggregation mentioned earlier.

A great number of additional techniques for aggregation characterisation at bulk levels exist in the literature, and commercial instruments are also available [17]. However, in microscale blood flows, and in particular in bifurcating geometries, where the flow and haematocrit conditions are not uniform, the local characteristics of aggregation play a major part in blood transport. For instance, it is still not clear if, and to what extent, RBC aggregation is beneficiary or harmful for the circulation [18]. The flux of aggregates of different sizes in the vasculature could reveal information about the transport of the formed structures under aggregating conditions.

To the best of our knowledge, there are no studies in the literature quantifying RBC aggregate flux characteristics in bifurcating microchannel flows. In this paper, we report on a study of RBC aggregate size fluxes in a T-junction for the first time, estimated through analysis of the bifurcating flows reported in Sherwood et al. [11,12], using the aggregate detection methodology developed by Kaliviotis et al. [14,15]. The paper is structured as follows: in Section 2 the technique for flux calculation is described and information regarding sample preparation, the flow system, and RBC aggregate size characterisation is provided; in Section 3 the analysis of the RBC aggregate size flux is presented for 34 different flow splits (i.e. 68 cases) together with an elaboration on the findings. The paper is closed with concluding remarks.

2. Methodology

Details for the experimental apparatus, sample preparation, flow measurement methodology and aggregate size characterisation can be found elsewhere [11,12,14] and only a brief description is provided here.

2.1. Sample preparation

Samples were acquired with the approval of the Southeast London Ethics Committee (ref: 10/H0804/21). Blood was collected from healthy volunteers into vacuum tubes (BD) preloaded with 1.8 mg/ml EDTA. The RBCs were washed twice in Phosphate Buffered Saline (PBS), centrifuged at 3000 rpm, and suspended in PBS containing D2000 (5 g/l). Dextrans are glucan polymers of various molecular weights that are widely used to induce aggregation between red blood cells. The aggregation dependency on Dextran concentration and molecular weight has been illustrated in various studies [19,20]. The haematocrit was adjusted to 25% by volume which is considered physiological for the microchannel dimensions used [21,22]. For consistency all experiments were conducted with a single sample.

2.2. The flow system

A microchannel (width $W = 100$ μm and depth $D = 40$ μm) was fabricated from SU8 using photolithography (Epigem, Redcar, UK), and is shown in Fig. 1. The sample was perfused through the parent branch of the microchannel by applying a pressure to the sealed inlet reservoir. The pressure in the inlet reservoir was controlled with a pressure control system comprising an actuated needle valve and a compressed nitrogen source. The fluid in the inlet reservoir was continuously mixed with a magnetic stir bar, in order to minimise the effects of RBC sedimentation. The stirrer was stopped when acquiring data.

The inlet reservoir pressure was set to a value resulting in a mean velocity of 320 μm s^{-1} in the parent branch. This velocity is physiological and comparable to the average cross-sectional velocities measured in the arterioles of the human conjunctiva (see for example the studies of Koutsiaris et al. [23,24]). The distribution of flow between the two daughter branches (flow split) was controlled by means of hydrostatic pressure difference by independently adjusting the height of the outlet reservoirs using micrometer stages. Between acquisitions, the channel was initially perfused at a high flow rate in order to ensure uniform hematocrit throughout the channel and system; subsequently the pressure was reduced to the desired level and 20 s were allowed for aggregation to reach a steady state before acquisition commenced [12].

2.3. Microscopy and micro-PIV system

An inverted microscope (Leica DM ILM, Germany) was used to visualise the flow, with the focal plane set to the centre of the

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