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Horse and dog blood flows in PDMS rectangular microchannels: Experimental characterization of the plasma layer under different flow conditions

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ABSTRACT

This work characterizes the plasma layer of horse and dog blood flows inside rectangular PDMS microchannels under different conditions (hematocrit of *ca.* 40% and 45%, respectively, flow rate varying between 1 and 30 μl/min, microchannel width and height varying from 100 to 350 μm and 30 to 40 μm, respectively), and relates it with the pressure drop. For that, images were acquired with a microscope/CMOS camera and digitally processed by a Matlab script. The results show that the cell-free layer thickness varies with the following parameters: it increases non-linearly with the increase of the blood flow rate (the average thickness growth observed with a 100% increase of the blood flow rate was *ca.* 55%); it increases with both microchannel width and height increase. Moreover, because plasma is a Newtonian fluid, the measured pressure drop varied linearly with the blood flow rate but with an apparent viscosity larger than that of plasma and smaller than that of the whole blood. This allowed inferring that plasma layers act as a lubricant making easier the blood flow. It was also observed a non-instantaneous response time, 3–4 s, of the erythrocytes to a squared-wave pulsatile flow, both when increasing and decreasing the flow rate.

1. Introduction

Artificial organs are designed to substitute anatomical organs in specific situations by guaranteeing their functions in extracorporeal blood circulation. Such are the cases of hemodialysers that perform separation functions of a failing kidney, or membrane blood oxygenators (MBOs) that replace temporarily shunted lungs in cardiopulmonary bypass during heart surgery, delivering oxygen to, and extracting carbon dioxide from blood.

One of the problems encountered in some types of MBOs is the low rate of O_2 mass transfer to the blood, which may be aggravated with the plasma layer build up at the blood/membrane interface [26,47]. That is why technical and medical progresses of MBOs have been accomplished by, among other improvements, the enhancement of mass transfer [22]. Reduction of the blood-side resistance to mass transfer has been achieved through the tentative disruption of such cell-free layer (CFL) by introducing mixing promoters over the flat sheet microporous membranes [10,22] or by intraluminal or complex extraluminal flow paths in hollow fibers modules [12,18,22], since it is well established that the efficiency of O_2 and CO_2 transport is dependent on the blood circulation conditions inside the membrane modules channels with characteristic dimensions typical of microfluidics. This problem has similitude with the limited delivery of oxygen to the tissues in microcirculation [43], which has been justified by the diffusional resistance in the capillaries [45] associated to the oxygen pressure gradient between erythrocytes and surrounding plasma, or by the plasma layer built up in microcirculation associated with the Fåhraeus effect [16] – see Tsai et al. [43] for further details.

Another problem associated with the use of MBOs is the plasma leaking [22], which may also be related with the hydrophilic character of the membrane and the cell-free layer build up at the membrane/blood interface, the latter constituting a barrier to gaseous mass transport. The strategies used to overcome this problem (e.g. use of silicone as a coating hydrophobic material) reduce even more severely the oxygen mass transfer [31,22].

In addition to the previous problems, blood is a quite complex fluid in both its constitution and flow structure. Because of that, and although pioneer studies on blood date back to Ancient Greece, when Greeks realized that such biological fluid formed distinctive layers when kept stationary in a recipient, and used their relative proportions as a diagnosis tool [36], it was only much later, in the 17th century and after overcoming technological limitations

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with the advent of microscopy, that studies of blood at smaller scales became possible. The scientific advances in different areas at microscopic scales did not stop ever since.

Particularly relevant was Poiseuille's work in the 19th century [42], who observed several phenomena on blood flows in the frog mesenteric microcirculation (venules and arterioles): the existence of a cell-free layer near the vessels walls, the plasma skimming process in bifurcations and the adherence of leukocytes to the vessels walls.

In the 20th century, in turn, the advent of MEMS (micro-elec tro-mechanical-systems) manufacture and, subsequently, micro-systems with fluids flowing within them launched microfluidics, which refers to flows of small volumes of fluids inside microdevices with characteristic lengths in the range 1–1000 μm having a huge prospective market [11,44,6,17,46,36].

The complexity of blood constitution lies in its concentrated suspension form of formed elements – erythrocytes or red blood cells (RBCs), leukocytes or white blood cells (WBCs), and platelets – in an aqueous solution, the plasma. This suspending solution exhibits a Newtonian behavior and contains numerous chemical species like proteins, glucose, clotting factors, inorganic ions, dissolved gases, hormones and other substances at low concentration. The non-Newtonian shear-thinning character of whole blood results from the red blood cells presence, particularly due to their large number density, aggregation tendency and deformation [38,50].

Understanding and characterizing blood flows *in vivo* (venules and arterioles) and *in vitro* (microchannels) is crucial to assess hemodynamic resistance and its regulation in microcirculation or in blood circulation inside extracorporeal devices like MBOs, and to analyze the associated mass transport processes. It should be stressed that the study of blood flows *in vitro* requires biocompatibility of the used materials. For this purpose, polymers have demonstrated to be particularly adequate and one of the most commonly used is the polydimethylsiloxane (PDMS) [27,21]. In fact, besides its hemocompatibility, PDMS is cheap, flexible and easy and fast to be used as raw material in the manufacture of microdevices [27,15].

Sharan and Popel [39] pointed out the phenomena that have been observed either *in vitro* or *in vivo* flows and that may be more or less relevant for blood circulation in artificial organs: (i) the Fåhraeus effect expressing the hematocrit dependence on vessel diameter [8,1,34,29], which may be important for the plasma leaking problem; (ii) the Fåhraeus–Lindqvist effect expressing the dependence of blood apparent viscosity on the vessel diameter [8,1,34,29], which may be of relevance for the required power to make flow circulate in the MBO; (iii) the existence of a CFL near the vessel wall, which is a known hemodynamic phenomenon well documented in the literature (e.g. [38,37,8,29,24,23,20,7] and strongly dependent on the characteristic dimension of the flow geometry, the flow rate and the hematocrit – the CFL effect is stronger when the characteristic dimension or the hematocrit are diminished or when the flow rate is increased [38,24,23,50,7], and this may be important for both the plasma leaking problem and to the mass transfer resistance to oxygen; (iv) the blunted velocity profile as a consequence of the RBCs aggregation at the low shear rate region, which indicates an increased local blood viscosity counterbalancing the lower viscosity values of the plasma layer region where shear rates are larger [38,37]; (v) the phase separation or plasma skimming, which nominates the disproportionate distribution of RBCs and plasma at vessel bifurcations [33,32,34,28].

The CFL formation has been attributed to the axial migration of RBCs and to their aggregation. The migration of RBCs toward the central flow region, which may be explained by a mechanical force imbalance acting on deformable suspended particles in low-Reynolds number flows with varying velocity gradients in the radial direction [14,19], provides the RBCs with a mean velocity greater than that of blood or plasma [8,25,29]. Aggregation is favored by both extrinsic factors, as the presence of agents in the suspending medium and mechanical shear, and intrinsic factors, like the cell type determined by species and age [20]. Even though, the interplay between cell migration and aggregation is complex [38,37,32,34]: at low shear rates, and in the presence of certain macromolecules like fibrinogen, RBCs aggregate in the form of 1D stacks-of-coins-like rouleaux or 3D aggregates [2,38,8,50,29], which enhances the formation of the plasma layer [38,37,8,32], but do not aggregate at large shear rates [38,37,8]. On the other hand, the process is reversible and disaggregation is possible and is determined essentially by mechanical effects – shear stresses [29].

From the previous discussion it appears that the blood flow characterization in microchannels, particularly as far as the CFL is concerned, can provide relevant information for the improvement of artificial organs design in avoiding plasma leakage and improving O_2 mass transfer by disrupting the CFL. Moreover, the characteristics of the plasma layer built up at the solid/fluid interface and their relation with the blood cells organization in the flow may be of capital importance, as they may contribute to improve the fluidity of the blood flowing in confined spaces by reducing the hydrodynamic resistance since plasma is a Newtonian fluid. In fact, it has been shown that increasing the plasma layer thickness reduces the blood effective viscosity [8,38,37], even in unsteady flow situations [1].

Those issues were addressed in the present work by characterizing the plasma layer in animal blood flows inside PDMS rectangular microchannels, as a function of the flow rate and geometry, and measuring the pressure drop in blood flows inside a rectangular cross-section microchannel at different flow rates, relating it with the plasma layer.

For that, images of the blood flows inside microchannels were acquired with a high-speed and high-resolution CMOS (complementary metal–oxide–semiconductor) camera, connected to a microscope. The images were then digitally processed with a procedure that makes possible the accurate assessment of the plasma layer thickness. Moreover, the geometry of the microchannels was also characterized by acquiring optical microscope images of their autopsies after use, together with SEM (scanning electron microscope) images analyses.

In addition, a pulsatile flow cycle was also studied herein in order to determine the horse blood flow relaxation time, *i.e.* to analyze the response time of the RBCs (or the plasma layer) to an instantaneous pressure stimulus.

2. Experimental

The experimental setup used herein for flow visualization, pressure drop measurements and the procedure to manufacture the used microchannels are described below. Moreover, the whole viscosity of the used blood is also characterized.

2.1. Microchannels manufacture and characterization

The microchannels used in the present work were manufactured with PDMS by soft lithography. This process is divided in two major steps: (i) the manufacture of the mold and, (ii) the manufacture of the microchannels.

Regarding the mold manufacture, an adequate resin, K-CL 100 from Kloé, France, was poured onto a rotating wafer (in a spin coater, Spin 150, SPS, Germany) and was subsequently exposed to UV light (UV-KUB2, Kloé, France) that changed it chemically. For such purpose, a photo mask with chromium cover containing the 2D