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Microconfined flow behavior of red blood cells

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A B S T R A C T

Red blood cells (RBCs) perform essential functions in human body, such as gas exchange between blood and tissues, thanks to their ability to deform and flow in the microvascular network. The high RBC deformability is mainly due to the viscoelastic properties of the cell membrane. Since an impaired RBC deformability could be found in some diseases, such as malaria, sickle cell anemia, diabetes and hereditary disorders, there is the need to provide further insight into measurement of RBC deformability in a physiologically relevant flow field. Here, RBCs deformability has been studied in terms of the minimum apparent plasma-layer thickness by using high-speed video microscopy of RBCs flowing in cylindrical glass capillaries. An in vitro systematic microfluidic investigation of RBCs in micro-confined conditions has been performed, resulting in the determination of the RBCs time recovery constant, RBC volume and surface area and RBC membrane shear elastic modulus and surface viscosity. It has been noticed that the deformability of RBCs induces cells aggregation during flow in microcapillaries, allowing the formation of clusters of cells. Overall, our results provide a novel technique to estimate RBC deformability and also RBCs collective behavior, which can be used for the analysis of pathological RBCs, for which reliable quantitative methods are still lacking.

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1. Introduction

In the microcirculation in vivo, red blood cells (RBCs) travel through microvessels with diameter smaller than the individual cell size in order to allow optimal gas transfer between blood and tissues [1]. The most important properties of RBC are deformability and aggregation. The high RBC deformability is mainly due to the viscoelastic properties of the cell membrane, especially shear modulus and surface viscosity [2]. Pathological alterations of RBC deformability are known to be implicated in several diseases, including diabetes, malaria, and sickle cell disease [3–6]. In light of such pathophysiological relevance, the measurement of RBC deformability has been the subject of a number of studies from single cell analysis (micropipette aspiration [7–9] and optical tweezers [10–12]) to flow techniques (ektacytometry [13]). Recently, microfluidic techniques [14], that are suitable to test a large number of cells in a physiologically relevant flow field, have been applied to design flow geometries resembling the microvascular network [15–18]. In such microconfined conditions, RBC shape departs from the classical biconcave geometry at rest by taking deformed configurations, resembling a bullet or a parachute, depending on flow rate and microvessel diameter [1,2,19]. The aggregation of RBCs is equally important, since the formation of *rouleaux*, dense 3D structures, plays a key role in the increase of whole blood viscosity at low shear rates, causing the non-Newtonian behavior of blood. Nevertheless, the mechanism of the formation of RBCs aggregates has not been fully elucidated.

In this work, a short review of our research focused on imaging-based in vitro systematic fluid dynamic investigations of RBC suspensions in microconfined conditions at the scale of single cells is presented. RBC membrane rheological behavior is investigated by analyzing the transient behavior of RBC shape in confined flow and by measuring the membrane viscoelastic properties in converging/diverging microchannels. RBC geometrical parameters, such as RBC volume, surface area, and distribution width (RDW), which is a measurement of the size variation and can be used as a significant diagnostic and prognostic tool in cardiovascular and thrombotic disorders [20], have been measured in microcapillary flow using high-speed microscopy. The obtained results provide a novel microfluidics methodology to measure RBC biomechanical properties, which are potential diagnostic parameters of altered cell deformability. In order to study the effect of reduced deformability of the RBC membrane, the flow behavior of glutaraldehyde (GA)-hardened RBCs has been analyzed, being GA a crosslinking agent of the proteins of the RBC membrane, able to change the viscoelastic properties of RBC

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membrane. The comprehension of the single cell behavior led to the analysis of the RBC flow-induced clustering.

2. Materials and methods

2.1. Blood samples

Fresh venous blood samples, drawn from healthy donors and used within 4 h from collection, are centrifuged to separate RBCs from white cells and platelets, and have been used following a procedure approved by the Ethics Committee of the University of Naples Federico II. Then RBCs are re-suspended in plasma and diluted with ACD anticoagulant and human albumin to a concentration of 1% for single cell experiments, or 10% by volume for clustering tests.

2.2. Experimental apparatus

The experiments are carried out either in 6.6 or 10 μm diameter glass microcapillaries placed in a flow cell and in a microfluidic device. The flow cell consists of two Plexiglass plates separated by a rectangular rubber frame. The glass microcapillaries lie on a cover slip sandwiched between the two Plexiglass plates, allowing observations with a high magnification oil immersion objective (100×). Regarding the microfluidic device, it is made of PDMS and is fabricated by using soft-lithography techniques with SU-8 as photoresist. The network pattern consists of a network of bifurcating channels of decreasing width (down to 10 μm), including converging–diverging flow sections, to mimic human microcirculation networks. Either the flow cell or the microfluidic device are placed on the motorized x–y stage of an inverted microscope, as shown in Fig. 1, and are connected by flexible tubing to a reservoir containing the RBC suspension, which has been fed by RBC by gravity or by using a micro-pumping system. Images of the flowing RBCs are acquired with a high-speed video camera (Phantom 4.3, operated up to 1000 frames/s), and are processed offline by a custom macro based on the library of a commercial software package.

In the majority of the experiments, the image analysis is fully automated. In fact, since cell size is comparable with the capillary diameter, RBCs lie essentially in only one focal plane. The first step of the image analysis consists in the selection of the images containing RBCs. In fact, it has to be said that most frames of the recorded movie could be empty, due to the low cell concentrations used in the experiments. Thus, only one cell at a time is found at most in the field of view. The selection of the frames containing RBCs is based on the comparison between the average gray level of each image to the background value. Once the RBC has been found, all the information needed (i.e. cell contour coordinates for velocity and surface area and volume measurements) will be extracted. For more rigorous analysis (i.e. in the case of RBC deformation index), the analysis of the selected cells is performed in a semi-automated way, manually choosing a gray level threshold to close the cell contour.

3. Results

3.1. Minimum apparent plasma-layer thickness

The observed RBC shape is shown in Fig. 2 as a function of cell velocity. In a microcapillary of diameter $D = 6.6$ μm all RBCs showed the typical axisymmetric parachute-like shape, tending to an asymptotic configuration at increasing cell velocity [21,22]. Asymmetric shapes are mostly observed (together with axisymmetric ones in an almost 1:1 ratio) in the 10 μm microcapillary, where the flow is still confined, causing RBC deformation, as shown in Fig. 3. Up to RBC velocity around 1 mm/s asymmetry is apparently the result of cell membrane folding.

At higher RBC velocities, asymmetric shapes are due to out-of-axis cell position (which is prevented in the smallest microcapillary by the

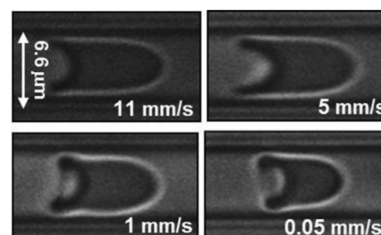

Fig. 2. Images of RBCs flowing in a 6.6 μm microcapillary at different velocities.

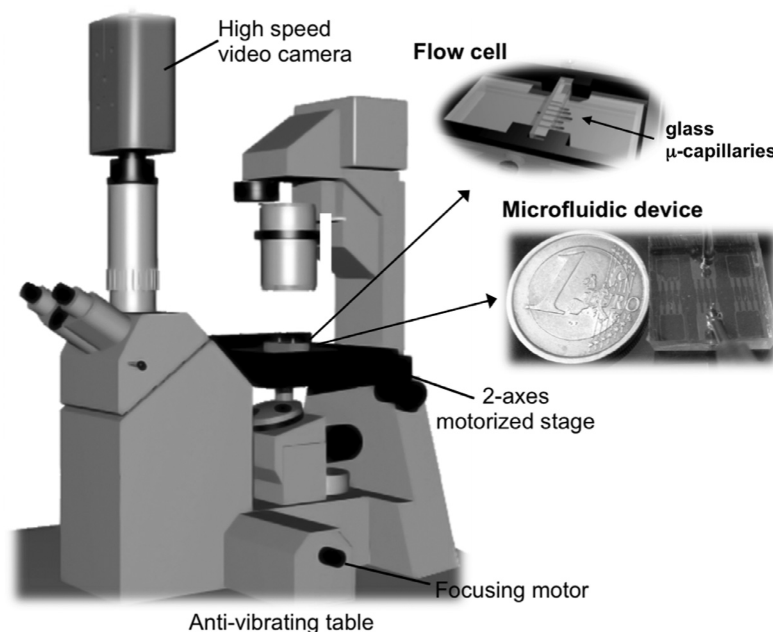

Fig. 1. Schematic of the experimental apparatus, with details of the flow cell with the 6.6 and 10 μm glass microcapillaries and of the PDMS microfluidic device.

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