Red blood cell dynamics, deformation and rheology via microfluidic experiments

Manouk Abkarian[*], Magalie Faivre[*], Annie Viallat[**] and Howard A. Stone[*]

[*] *Division of Engineering and Applied Sciences, Harvard University, Cambridge, MA, 02138*

[**] *Laboratoire de Spectrométrie Physique, UMR 5588, Université J. Fourier, BP 87, 38402 Saint Martin d'Hères cedex, France*

Summary Blood is principally made of a calibrated suspension of red blood cells (RBC). Depending on the flow, RBCs move alone or in aggregates (rouleaux), often in capillaries of smaller sizes than their own radius. Blood is an "intelligent" fluid, as its rheological properties adapt according to the different situations of the flow. This capacity, which is not fully understood, comes partly from the significant deformability of RBCs. The understanding of the flow behavior of a single confined RBC is a first step to understand the basic rheological properties of the blood in arteries. To achieve this goal, we used microfluidic technology as a tool to explore hydrodynamics of a single cell and the hydrodynamic interactions among cells. We are also using such measurements to infer the individual mechanical properties of their membrane.

The aim of this paper is to present preliminary experiments of the motion of red blood cells in microfluidic channels mimicking the circulation in microvessels. We want to explore the role of the deformability of red blood cells on their motion in confined geometries such as capillary vessels. For example, we are interested to know how deformability can affect, or even assist, the formation of rouleaux of red blood cells (stacks of several red blood cells), which are believed to reduce the apparent viscosity in small capillaries [1,2]. Indeed, accounting for basic molecular forces (e.g. adhesion between proteins, depletion forces, etc.) the formation of rouleaux has been extensively studied in quiescent blood; such forces cause or prevent aggregation and can explain the elastic properties of coagulated blood [3,4,5,6]. Nevertheless, the formation of rouleaux is a dynamical process, as it occurs during the flow of blood in arteries. It is difficult to understand how molecular or depletion forces could play a role on the first stage of the aggregation of red blood cells and therefore, we study experimentally the effect of deformability and hydrodynamic interactions with the walls and among cells to explain the first steps of the rouleaux formation. To the best of our knowledge, these are some of the first, if not the first, detailed measurements focusing on the interplay between cell deformability, confinement, and hydrodynamic interactions. Finally, we are exploring the mechanical properties of red blood cells by using microfluidics as a measurement tool.

The technique that we use to produce the microchannels is the well-known technique of soft lithography [7,8]. The method consists of producing a negative relief of the channels in poly(dimethylsiloxane) (PDMS), curing the prepolymer (Sylgard 184, Dow-Corning) on a Si-wafer master having the positive relief of the microchannels, which themselves are formed with a photoresist (SU 8-2 to SU 8-50, Microchem). This PDMS membrane is peeled off the SI wafer and applied against a glass microscope slide previously oxidized (with the PDMS membrane) in a plasma chamber. High-speed video is used to follow the motion and deformation of the cells.

HYDRODYNAMICS INTERACTIONS BETWEEN DEFORMABLE RED BLOOD CELLS

Interaction of red blood cells in 1D channels

To analyse the interactions among red blood cells, we made very small and square channels (5μm x 5μm in cross-section), which are smaller than the red blood cell size. During flow, the red blood cells flow in a single file and are forced to deform into a parachute-like shape, which is commonly already referenced in the literature [9,10]. We refer to this configuration as "1D". Our interests are in the hydrodynamic interactions between the cells, which to the best of our

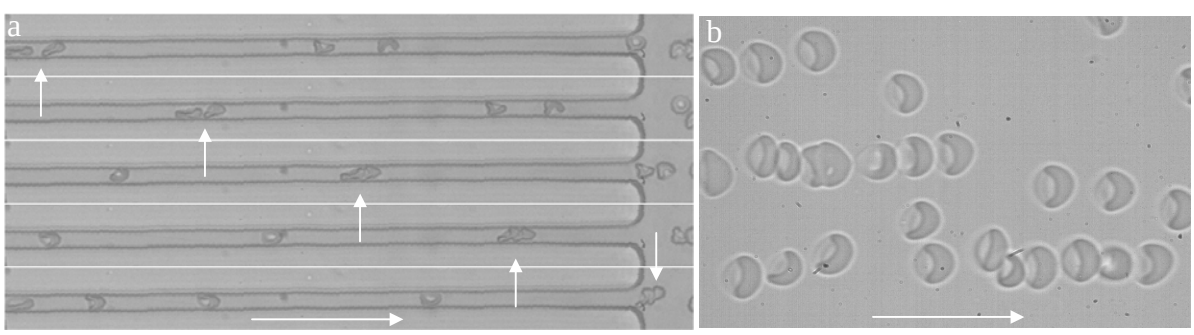

Figure 1. (a) Sequence of the motion and the deformation of two red blood cells (indicated with small white arrows) in a microchannel. The cross section of the channel is 5μm x 5μm in cross-section. The time between two frames is 100ms and the large white arrow represents the direction of the flow. (b) Red blood cells flow in a Hele-Shaw type channel (100 μm wide and 5μm high). We can observe on the figure the formation of files of red blood cells aligned in the direction of the flow.

knowledge have not been experimentally measured. In Figure 1a we show the results of several preliminary experiments highlighting the hydrodynamic interactions, which lead to eventual aggregation, that occur as the cells flow along the

channel. Basic results of low-Reynolds-number hydrodynamic argue that cell deformability is necessary to account for the kinds of interactions shown in the Figure 1a; some part of this effect may be due to changes of mobility of the cell owing to deformation during flow. Detailed measurements are in progress to completely characterize this hydrodynamic interaction and will be compared with theoretical calculations.

Interaction of red blood cells in 2D channels

In a second set of experiments we relaxed the constraint of the walls in one direction. The flow is produced in plane channel (100μm x 5μm section), or Hele-Shaw cell, where the same average flow is maintained across the channel. We refer to this flow configuration as "2D" and it is shown in Figure 1b. We observe that an initially uniform suspension of red blood cells arrange themselves into files, which is clearly a precursor of rouleaux formation. Obviously, hydrodynamic interactions are responsible for this pattern formation. Detailed measurements are in progress to understand the dynamical progression of the rouleaux formation.

ENTRANCE AND EXIT OF RED BLOOD CELLS IN MICRO-CHANNELS

In a third set of experiments, shown in Figure 2, we are focusing on the time-dependent shape changes that occur when a red blood cell enters or exits a small channel. The deformation is related to mechanical properties of the membrane. For example, the "entrance length" over which the cell adopts a steady shape has been numerically calculated [11,12] and a comparison of our experiments with the results of simulations should allow the extraction of mechanical properties of the membrane.

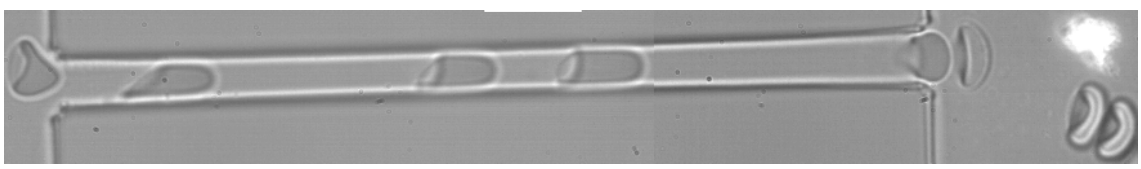

Figure 2. Entry and exit of red blood cells from a 1D micro-channel: We can see on the figure the different steps of the deformation: first the entry where the red blood cell deforms until it reaches a stationary shape in the channel. Then, the exit process that is different from the entry and presents totally different deformation features.

CONCLUSIONS

In conclusion we have indicated three sets of on-going experiments, which probe dynamical response and mechanical properties of deformable red blood cells. To the best of our knowledge, these are some of the first, if not the first, detailed measurements focusing on the interplay between cell deformability, confinement, and hydrodynamic interactions. By using microfluidic techniques, we are also using these ideas in reverse by making microfluidic devices for biomedical applications that allow us to check the mechanical properties between healthy and sick red blood cells.

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