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Blood flow and microgravity

Écoulement sanguin et microgravité

Lionel Bureau [a,b], Gwennou Coupier [a,b], Frank Dubois [c], Alain Duperray [d,e], Alexander Farutin [a,b], Christophe Minetti [c], Chaouqi Misbah [a,b,*], Thomas Podgorski [a,b], Daria Tsvirkun [a,b,d,e,f], Mikhail Vysokikh [f]

[a] *Université Grenoble Alpes, LIPhy, 38000 Grenoble, France*
[b] *CNRS, LIPhy, 38000 Grenoble, France*
[c] *Service de chimie physique EP, Université libre de Bruxelles, 50, avenue Frankin-Roosevelt, CP16/62, B-1050 Brussels, Belgium*
[d] *INSERM U1209, Institut Albert-Bonniot, 38000 Grenoble, France*
[e] *Université Grenoble Alpes, IAB, 38000 Grenoble, France*
[f] *Research Center for Obstetrics, Gynecology and Perinatology, 4, Oparin street, Moscow, 117997, Russian Federation*

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ABSTRACT

The absence of gravity during space flight can alter cardio-vascular functions partially due to reduced physical activity. This affects the overall hemodynamics, and in particular the level of shear stresses to which blood vessels are submitted. Long-term exposure to space environment is thus susceptible to induce vascular remodeling through a mechanotransduction cascade that couples vessel shape and function with the mechanical cues exerted by the circulating cells on the vessel walls. Central to such processes, the glycocalyx – *i.e.* the micron-thick layer of biomacromolecules that lines the lumen of blood vessels and is directly exposed to blood flow – is a major actor in the regulation of biochemical and mechanical interactions. We discuss in this article several experiments performed under microgravity, such as the determination of lift force and collective motion in blood flow, and some preliminary results obtained in artificial microfluidic circuits functionalized with endothelium that offer interesting perspectives for the study of the interactions between blood and endothelium in healthy condition as well as by mimicking the degradation of glycocalyx caused by long space missions. A direct comparison between experiments and simulations is discussed.

RÉSUMÉ

L'absence de gravité lors de longues missions spatiales peut altérer le fonctionnement cardiovasculaire à cause, en partie, de l'absence d'activité physique. Ceci a des répercussions sur l'hémodynamique, et en particulier sur le niveau de contraintes de cisaillement auxquelles sont soumis les vaisseaux sanguins. Un séjour de longue durée dans l'espace peut conduire à un processus de remodelage vasculaire via une cascade complexe de mécanotransduction qui couple la morphologie des vaisseaux et leur fonction aux signaux mécaniques dus au passage des corpuscules sanguins le long des parois vasculaires. Dans

* Corresponding author.
E-mail address: chaouqi.misbah@univ-grenoble-alpes.fr (C. Misbah).

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ces processus, le glycocalyx – brosse de biopolymères épaisse d'environ un micromètre, tapissant la paroi endothéliale et directement exposée au flux sanguin – joue un rôle central dans la régulation des interactions mécano-biochimiques. Dans cet article, nous présentons des résultats expérimentaux obtenus en microgravité concernant la force de portance s'exerçant sur les globules rouges et sur les vésicules ainsi que les mouvements collectifs, puis quelques résultats préliminaires portant sur la fonctionnalisation de circuits artificiels par des brosses de polymères et par des cellules endothéliales. Ceci offre des perspectives intéressantes pour étudier l'interaction entre écoulement sanguin et endothélium, sain ou altéré à la suite d'une dégradation du glycocalyx mimant les effets de longues missions spatiales. Une comparaison directe entre expériences et simulations sera présentée.

1. Introduction

Blood represents a very challenging fluid medium in terms of theoretical description because of the scale-dependent changes of its properties and its complex mechanical and biochemical structure. Pathologies associated with blood flow and cardiovascular functions are the primary cause of mortality in Europe and the USA. Intense multi-disciplinary research is therefore essential to develop innovative approaches to identify the key elements that promote cardiovascular disorders.

A major factor that affects cardiovascular dysfunctions is the absence of gravity during stays in space. It is well known that a long-term mission in space is associated with cardiovascular dysfunctions. For example, heart rhythm disturbances have been seen among astronauts. It is documented that long space missions lead to reduction of plasma volume, as well as left ventricular mass decrease. To give a simple estimate that the absence of gravity should have a clear consequence on blood flow, let us evaluate the pressure due to gravity at the level of the heart and compare it to that of the internal body blood pressure. The first is given typically by $\rho g h$. Taking density ρ to be that of water and g earth gravity and considering that the heart height in the upright position is of about $h = 1$ m, we obtain 10^4 Pa $= 0.1$ bar. An average blood pressure is, in terms of medical common usage, 14/8, which means an extra pressure (in comparison to atmospheric pressure) of 140 mm of mercury in the systolic regime and 80 mm in the diastolic regime. Given the fact that the atmospheric pressure corresponds to 760 mm of mercury, a blood pressure of 14/8 thus corresponds to the range of 0.1–0.18 bar. Interestingly the pressure related to earth gravity is very close to the body internal extra pressure. One expects thus that a long term mission in microgravity should impact physiological functions, that can potentially lead to cardiovascular anomalies.

Besides myriads of macroscopic studies (heart rhythm, plasma volume, blood vessel remodeling), other measures performed on astronauts after long missions in space reported several microscopic disturbances. For example it has been reported that space missions are accompanied with anemia, hemolysis and with an increase of amylase activity [1] as well as with variations in Red Blood Cell (RBC) membrane phospholipid composition [2]. Amylase is an enzyme that is known to digest sugar molecules, which are abundant on the glycocalyx as well as on the RBC surface. It is thus an essential goal for studies on blood flow to analyze the far reaching consequences of the impact of microgravity on blood flow, which is our long-term objective.

For many years we have been involved in trying to extract the basic elementary blocks that govern the blood flow properties, ranging from the study of single cell dynamics up to collective motions. Other studies consist in functionalizing artificial circuits with polymer brushes and more recently with endothelial cells in order to mimic real blood vessels. In particular an objective is to alter the endothelium, thanks to enzymatic digestion (mimicking the amylase activity in space), and to study the related consequences. In what follows, we shall briefly describe the main achievements of our studies and the various microgravity experiments that have allowed us to extract this information. In particular we shall discuss the lift force on vesicles and on RBCs, experiments performed in parabolic flights where lipid vesicles are simplified models for RBCs. It will be seen that microgravity has offered a unique opportunity to analyze this question and that an analytical theory as well as full numerical simulations provide a very good agreement with experimental observations. We shall discuss the dynamics of suspensions obtained from experiments in sounding rockets, and explain the inhomogeneous distribution of the suspension in the channel by referring to the knowledge gained from the study of lift force. Also it will be shown that a bidisperse (two vesicle sizes) suspension exhibits a segregation triggered by lift force and shear-induced diffusion: small vesicles are pushed towards the periphery, while large ones have the tendency to migrate towards the center. This is reminiscent of the margination effect known in blood flow, where platelets (small size cells as compared to RBC) are marginalized toward the walls of the blood vessels. Regarding endothelium, we have first clarified the role of a polymer brush (mimicking the glycocalyx) on solvent flow and RBC dynamics, and have found quite significant and striking effects. Those effects were also revealed in our recent simulations. We shall briefly discuss the achievement of artificial microfluidic circuits coated with endothelial cells, with which we have performed the first successful preliminary experiments on blood flow in these functionalized circuits.

2. The studied systems

2.1. Experiments

The studied systems are phospholipid vesicles and RBCs. Vesicles are produced from dioleoylphosphatidylcholine using the well-known electroformation technique. They encapsulate a sucrose in 1:4 glycerol–water mixture, to which dextran can be added to increase the inner fluid viscosity. Samples were then diluted in an outer medium (glucose in a 1:4 glycerol–water mixture). The viscosity ratio λ between the inner fluid and the outer fluid is an important control parameter, which was varied between 1 and 6.5.

Blood samples were provided by the CHU ('Centre Hospitalier Universitaire') of Grenoble, France, from hematologically healthy donors. Phosphate-buffered saline (PBS) solution, which has a viscosity close to that of plasma, was used as the suspending medium for the RBCs that had been extracted from whole blood by centrifugation. Addition of dextran in the suspending medium allowed us to study the effect of fluid stress on the lift of RBCs through the increase in external fluid viscosity.

Lift and diffusion experiments were carried out in a parallel-plate shear chamber, which is described in [3], with a gap of 170 μm. The three-dimensional positions of the vesicles and RBCs are captured by digital holographic microscopy, a specific tool that allows us to get, in particular, the cell position along the optical axis, which is also the transverse migration direction. Details of the method used for vesicle detection are reported in [4]. The procedure was adapted for RBC detection [5].

Bio-inspired microchannels are designed according to two different strategies: (i) We have first used synthetic polymer brushes, *i.e.* layers of end-tethered macromolecules, grafted on the inner walls of glass capillaries, in order to study, using high-speed videomicroscopy and particle-tracking velocimetry, the effect of such a surface-bound soft layer on fluid flow [6] and single-file dynamics of RBCs [7]. (ii) Very recently, we have started to design more realistic devices that consist in standard microfluidic channels, with a cross-section of a few hundreds of μm^2, in which we seed and culture endothelial cells (Human Umbilical Vein Endothelial Cells, HUVEC) that adhere to the channel walls and eventually form a full monolayer lining the lumen of the microchannels.

The project on endothelial dysfunction grew up thanks to various fruitful discussions with Bernard Zappoli, during the preparation of the 'Séminaires de prospectives du CNES' (Biarritz 2009); he had the early vision to elaborate with one of us (C.M.) a project that would allow us to ultimately go towards deeper diagnosis during long-term space missions. The endothelial dysfunction constitutes a prelude to cardiovascular dysfunction and disease, and its detection goes much beyond the presently available medical diagnosis. On the one hand, it is hoped that thanks to *in vitro* studies, we will be able to identify the key elements associated with anomalies of blood flow due to endothelial dysfunctions. On the other hand, thanks to non-invasive photoacoustic techniques, developed presently at LIPhy, we shall be able to monitor *in vivo* the occurrence of thromboembolisms triggered by endothelial dysfunction.

2.2. Theory and simulations

Due to the smallness of vesicles and RBCs (radius from 5 to 20 μm, and 3 μm, respectively) and the involved velocities (lower than or of about 1 cm/s), the Reynolds number is small, and the encapsulated as well as the ambient fluids are described by the Stokes equations. At the membrane, we impose: (i) force balance, that is the jump in hydrodynamics stress across the membrane is counterbalanced by the membrane force, including bending and shear elasticity associated with the cytoskeleton [8], (ii) continuity of velocity, and (iii) membrane incompressibility. The Stokes equations and the boundary conditions are a sufficient set to describe fully the shape dynamics of vesicles and RBCs. This set can also be converted into an integral equation (based on the Green's function techniques) [9] for the membrane velocity, which has been so far intensively used for vesicles and capsules [10–17]. The numerical details can be found in [14,8]. The analytical theory is based on an expansion of the shape in spherical harmonics [18,19]. This theory has allowed several progress as well as identifying interesting regions of the parameter space. Reviews on the progress achieved in this field can be found in [20–26].

3. Microgravity experiments: lift and diffusion of vesicles and red blood cells

A classic result in low-Reynolds number hydrodynamics is that the migration of spherical particles transversally to the direction of the flow is prohibited by the linearity and time-reversal symmetry of the Stokes equation. However, the deformability of vesicles and RBCs allows a symmetry breaking that may lead to transverse migration, be it due to interactions with walls or with neighboring cells. The consequence of the first interaction will be the apparition of a depleted layer near the walls, while the second interaction has a reverse effect, as it tends to widen the cell distribution. As a result of these two opposite effects, the stationary state for the suspension is a distribution that has a maximum in the center and vanishes near the wall. Although the existence of a cell-free layer in the blood circulation is well known since the pioneering work of Poiseuille [27], the lift force on the RBCs is poorly documented, in particular from the experimental viewpoint, except by some papers in the 1970s [28]. This is probably due to the complexity of RBC dynamics under shear flow, and to the effect

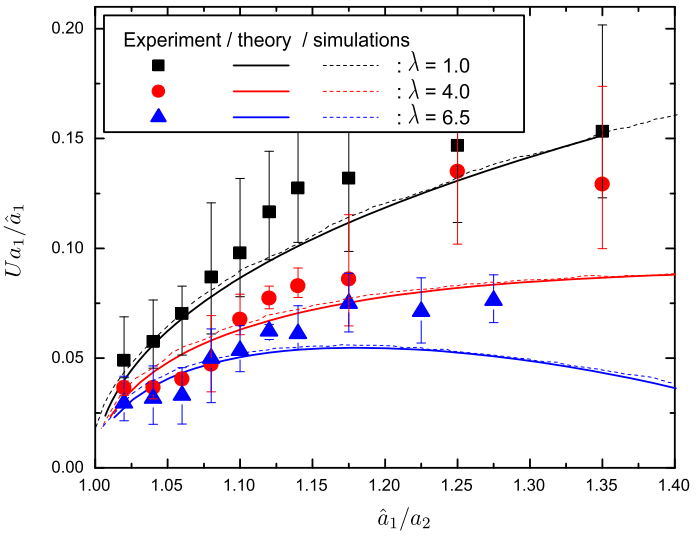

Fig. 1. Amplitude of the lift velocity for three different viscosity contrasts. The measured lift velocity is $Ua_1/\hat{a}_1$, where a_1 is the length of the long axis of the vesicle and $\hat{a}_1$ the length of its projection along the shear gradient direction, which is the optical axis. The apparent aspect ratio is the ratio between $\hat{a}_1$ and the width a_2 of the vesicle in the vorticity direction. The solid line for $\lambda = 1$ is an analytical theory obtained in [33].

of sedimentation velocity, which is of the order of 1 μm·s^{-1} in physiological conditions and thus easily screens lift effects. The lift force has been also analyzed for other types of cells than RBCs [29].

In parabolic flight experiments, we used microgravity, not only to remove this screening effect, but also the normal gravity phases to control the initial conditions for the whole sample by letting the cell sediment on the bottom plate so as to study, under microgravity conditions, how they lift away from that wall.

As predicted by Olla [30], the lift velocity of a lipid vesicle in tank-treading regime can be written as $\dot{z} = U\dot{\gamma}R^3/z^2$, where z is the distance of the cell center of mass from the wall, R is the typical cell size, $\dot{\gamma}$ is the shear rate and U is a dimensionless factor that depends on the cell's shape and mechanical properties, that is, on the very detail of the cell's reaction to flow stress. This result holds for $\lambda = 1$, as we previously reported [3], but also for higher viscosity contrasts, as we report here for the first time. Following the analysis described in [3], we report in Fig. 1 the dependency of U on the apparent aspect ratio of the cells, which shows good agreement with our theory and our numerical simulations. It can be seen that lift velocity decreases when λ increases and that, at high values of λ, it can even drop down to zero upon high deflation of the vesicles. Those features where already observed in Poiseuille flow [31] and can be related to the decrease in the inclination angle of the cell relatively to the flow direction when λ or its aspect ratio increases. The lift force has been revisited in [32] and in [33]. This has allowed us to obtain a better agreement with experiments as reported in Fig. 1.

In Ref. [5], we showed that despite being in tumbling regime at physiologically relevant shear rates, RBCs follow the same law for the lift velocity, with a dimensionless lift parameter U that is also a decreasing function of λ. In particular, at physiological viscosity contrast, UR^3 is in the order of 0.4 μm^3. Consequently, if the cell is slightly above the wall so that $z \simeq 5$ μm, one finds $\dot{z} \simeq 2$ μm·s^{-1} for the typical shear rate $\dot{\gamma} = 100$ s^{-1}, a value that is comparable to sedimentation velocity.

As they lift away from the wall, vesicles may also interact with each other and the initial thickness of the suspension, which is close to cell size as all cells had sedimented, will increase. If one makes the assumption that lift effects and interaction effects act independently on a vesicle, we can extract information on the diffusion coefficient associated with the multiple interactions between cells from the time evolution of the distribution thickness. Since all effects depend on cell size, sorting out the produced vesicles by size turned out to be necessary, as electroformation produces a highly polydisperse suspension [4]. This was achieved thanks to the Pinched Flow Fractionation technique, which relies only on hydrodynamic effects that are controlled in a microfluidic chip [34]. One can find in the literature principles of monodisperse vesicle production [35,36], although we are not aware of the use of such samples under flow, which may be related to stability issues. With the sorting method we developed, a partial polydispersity in size and reduced volume remains, which may account for the dispersion seen in the results. In Fig. 2, we show the time evolution of sorted vesicles with $\lambda = 1$ in a parabolic flight experiment (see also Ref. [4]). Assuming that the mean height is controlled by the lift, one can consider the standard deviation of the distribution and relate it to the shear-induced diffusion coefficient.

The diffusion equation in the absence of transverse lift reads $\partial_t\phi = \partial_z(D\partial_z\phi)$, where $\phi(z,t)$ is the cell volume fraction, assuming the cell distribution is uniform in the flow and vorticity directions. In the semi-dilute regime, the diffusivity $D = f_2R^2\dot{\gamma}\phi$ is proportional to the frequency of pair interactions $\dot{\gamma}\phi$, a straightforward scaling for shear-induced diffusion due to pair interactions [37,38]. f_2 is a dimensionless parameter to be determined, which depends on the detail of the interaction between cells. For a constant amount of cells, this equation admits a self-similar solution (to which other initial distribution should converge), which has the shape of a parabola [39,5]. From that, the standard deviation in the cell distribution can

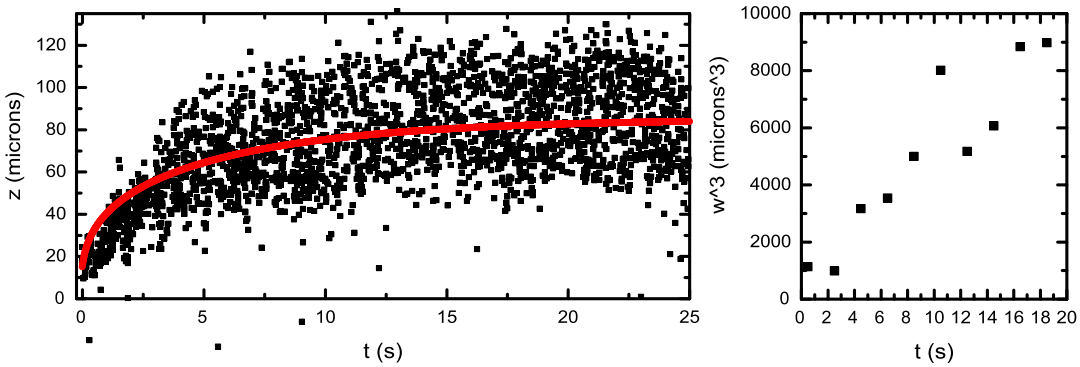

Fig. 2. Left: z positions of vesicles across the shear chamber of thickness $h = 170$ µm (parabolic flight experiment). The shear rate is 50 s^{-1} and $\lambda = 1$. The mean volume concentration is 7.2×10^{-3}. Vesicles were sorted by the pinched flow fractionation method, so that $\hat{a}_1 = 16 \pm 3$ µm. Only vesicles with apparent aspect ratio between 1.1 and 1.3 are represented. Quasi-spherical vesicles lift much less. More deflated vesicles are rare. Each dot corresponds to one detected vesicle. The full line shows the one-parameter fit by the $z(t)$ evolution obtained by solving $\dot{z} = A(1/z^2 - 1/(h-z)^2)$, to take into account the effect of both walls. Right: w^3 as a function of time, where w is the standard deviation of the distribution.

be calculated, and one finds $w(t) = w_0(1 + \frac{9N_0 f_2 R^2 \dot{\gamma}}{10\sqrt{5} w_0^3} t)^{1/3}$. w_0 is the standard deviation at $t = 0$ and $N_0 = \int \phi \, dz$ is a constant. The exponent 1/3 indicates that we deal with a subdiffusive process, which can be understood from the fact that diffusion is due to interaction with neighbors, which become less and less numerous as time passes by. As seen in Fig. 2, this subdiffusive scaling is found in our experiment, and we find $f_2 = 0.069 \pm 0.023$, where ± 0.023 corresponds to the 95% confidence interval. Note that, in a first approximation, it can be considered that this coefficient should not depend too much on the vesicle's aspect ratio, the case of spherical vesicles being put apart, as we discussed in a study on the detail of pair interactions between vesicles [40]. The error on the determination of f_2 is mainly due to noise in the experiment, due to the smallness of the values for the standard deviation compared to the possible drifts due to residual gravity fluctuations or device vibration, and to the accuracy of the cell position detection, which is in the order of 1 µm. However, this result could be confirmed in sounding rocket experiments, where vibrations and gravity fluctuations are expected to be much lower.

Our experiment participated in the Maser 11 and Maser 12 campaigns organized by ESA. Sounding rocket experiments do not allow the control of initial positions by sedimentation, but allow for longer time of microgravity, which gave us the possibility to observe the stationary vesicle distribution between the two plates of the shear chamber. As this distribution is the result of the balance between the lift force (already well characterized) and the shear-induced diffusion, it allows us to determine f_2. For the Maser 12 campaign, we considered three samples of vesicles of different sizes and $\lambda = 1$. Suspensions of large, medium, and small vesicles were successively injected. The radii of these populations lie respectively in the ranges [22; 35], [15; 20], and [5; 15] µm. In addition, two concentrations of the medium-size population were considered ($\langle\phi\rangle = 0.0031$ and $\langle\phi\rangle = 0.0016$), while the concentration of the large-size population was $\langle\phi\rangle = 0.0014$. The suspensions were sheared at 50 s^{-1}. It turned out that this was not sufficient to reach the stationary distribution for the small vesicles within the experimental time dedicated to this experiment (around 30 s). For the other populations, the stationary distribution can be obtained by balancing lift and diffusion volume flux [41]: $J_L + J_D = 0$, where $J_L = \phi U \dot{\gamma} R^3 \left(\frac{1}{z^2} - \frac{1}{(h-z)^2}\right)$ is the flux due to lift forces and $J_D = -f_2 \Phi \dot{\gamma} R^2 \frac{\partial \phi}{\partial z}$ is the diffusive flux.

The solution is $\phi(z) = \max\left(0, A - \frac{URh}{f_2 z(h-z)}\right)$, where A can be determined by considering the known injected concentration of vesicles. f_2 can then be determined from the one-parameter fit of the distribution by the theoretical solution, taking for U the mean value for the vesicle population considered here (apparent aspect ratio between 1.02 and 2), which was given by our previous theoretical calculations. From these three experiments, we found $f_2 = 0.063 \pm 0.024$. This is consistent with our previous finding.

Finally, when injecting two sizes of population, as in Fig. 3, one finds that the small vesicles are expelled from the center, as a result of both their weaker lift and the asymmetry in their interaction with large ones. This segregation phenomenon is similar to platelet margination in blood flow, a complex mechanism that has been numerically studied in some recent papers (see, e.g., [42]).

4. Flows in brush-coated and endothelium-bearing microchannels

It is recognized that, beyond its biochemical and mechanotransduction functions, the endothelial glycocalyx plays a hydrodynamic role and affects the resistance to blood flow, in particular in microvessels whose dimensions are comparable with the size of RBCs. However, *in vivo* experiments are extremely challenging to perform, and control over the flow parameters and the actual state and thickness of the glycocalyx is limited [43]. This calls for *in vitro* fundamental studies,

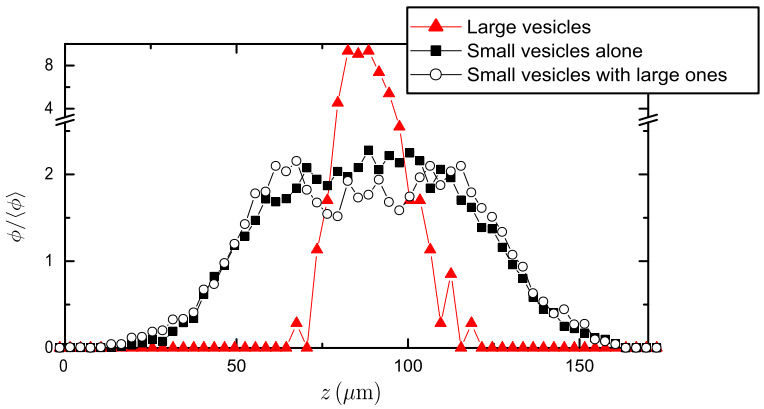

Fig. 3. Volume fraction renormalized by the mean value for two experiments: small vesicles alone ($R \in [5; 15]$ and $\langle\phi\rangle = 0.0025$), and small vesicles ($R \in [5; 15]$ and $\langle\phi\rangle = 0.0016$) mixed with large ones ($R \in [20; 30]$ and $\langle\phi\rangle = 0.00077$).

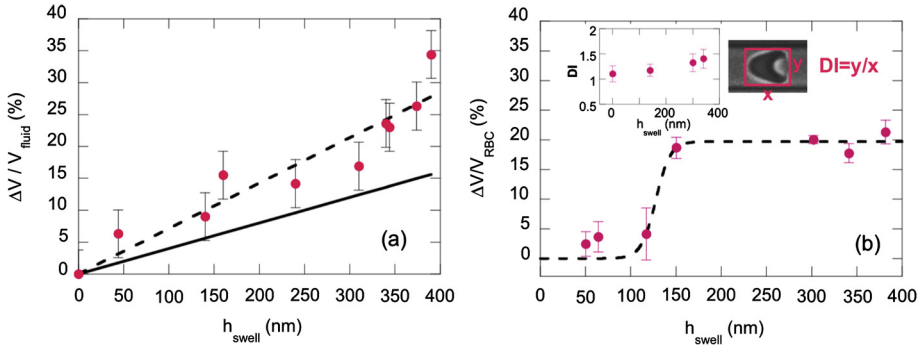

Fig. 4. (a) Relative reduction of the mean fluid velocity as a function of brush swollen thickness h_{swell}. Symbols are experimental measurements, the continuous line is the prediction assuming no flow in a layer of thickness h_{swell}, and the dashed line is the best linear fit to the data. (b) Relative reduction of the RBC velocity as a function of h_{swell} (the dashed line is a guide for the eye). Inset: (right) definition of the RBC deformation index (DI) as the aspect ratio of a box bounding the cell, (left) evolution of DI with h_{swell}.

in which the hydrodynamic effect of a surface-bound soft and deformable macromolecular layer can be more easily and systematically studied.

In this spirit, we have elaborated microchannels made of cylindrical glass capillaries with an inner diameter of 10 µm, in which we have grown, using the so-called grafting-from method, polymer brushes that are swollen in aqueous media and mimic the presence of the glycocalyx [6]. This strategy allows us to have a good control over the imposed flow conditions as well as over the thickness of the surface-bound layer.

We have thus shown that, in the presence of a polymer brush, the hydraulic resistance of a microchannel increases, *i.e.* the flow velocity is reduced, all the more so that the brush thickness is important, as illustrated in Fig. 4a. Interestingly, we find that for brush thicknesses comparable with that of an actual glycocalyx (several hundreds of nanometers), the observed velocity reduction, in the order of 30%, closely compares with what has been deduced from *in vivo* measurements [43]. This suggests that our bio-inspired microchannels do allow us to reproduce correctly the hydrodynamic effect attributed to the glycocalyx. Still, we note that the magnitude of this effect is systematically larger than expected from a mere reduction of the inner channel diameter due to the presence of the brushes (see Fig. 4a). Although we cannot yet account quantitatively for such an unexpectedly large impact of the wall-bound brushes, recent numerical simulations provide evidence for the development of non-trivial near-wall backflows and for the build-up of surface waves at the brush/fluid interface [44], which might be at the origin of our observations. This points to the existence of an extremely rich phenomenology in such problems of flow with soft boundaries.

Using the same type of functionalized channels, we have also studied the dynamics of RBCs. We have observed that, in contrast to the previous situation of pure fluid flow, the effect of a polymer brush on RBC velocity becomes significant only above a threshold in brush thickness (around 150 nm), as shown in Fig. 4b. Even more puzzling, we find that the RBC velocity reduction is independent of brush thickness above the threshold (Fig. 4b), while the RBC deformation seems to be a monotonously increasing function of brush thickness (Fig. 4b, inset). This suggests the existence of some sort of velocity selection mechanism, by which RBCs tend to adjust their shape in order to maintain a constant velocity, even though the polymer brush occupies a larger and larger fraction of the channel diameter. These observations fully remain to

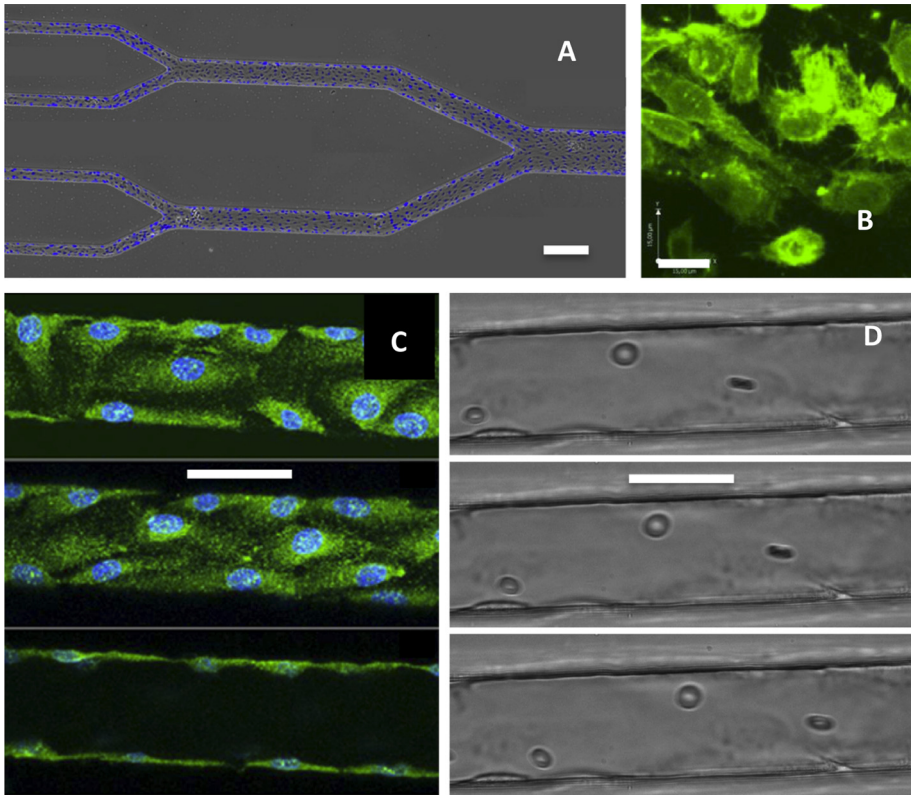

Fig. 5. A: A HUVEC monolayer cultured inside the branched structure of the designed circuits; overlay of a bright field image with a fluorescent one revealing the nuclei (stained with Hoechst and displayed in blue). Scale bar: 200 µm. B: Confocal fluorescence image showing the presence of the glycocalyx on the cell surface (stained by WGA-FITC, in green). Scale bar: 15 µm. C: Confocal fluorescence images showing endothelial cells at the bottom (upper image), top (middle image) and side (lower image) walls of the channel (cytoplasm in green, nuclei in blue). Scale bar: 40 µm. D: Image sequence showing RBCs flowing, from left to right (flow direction) and from top to bottom (time direction), in a channel covered by a HUVEC monolayer (seen as protrusions on the side walls). Scale bar: 40 µm.

be elucidated at that stage, but indicate that the RBC dynamics in the presence of a polymer brush is not simply controlled by the behavior of the suspending fluid, and most likely involves more subtle cell/brush interactions.

The latter issue regarding RBC/brush interactions also points to the fact that, in the above studies, we have mimicked the endothelial surface layer using brushes of neutral polymers. This can be considered as a crude approximation, since an actual glycocalyx is mainly composed of polysaccharides, *i.e.* charged biomacromolecules, which are likely to play an important role in the details of physicochemical interactions. In order to develop microfluidic devices that more closely mimic physiological situations, we have recently adopted a different approach. Building on a recent experimental work [45], we have designed networks of square section microchannels made of polydimethylsiloxane (PDMS), into which we have seeded and cultured HUVEC (Human Umbilical Vein Endothelial Cells) until the formation of a confluent cell monolayer lining the four walls of the microchannels, as illustrated in Fig. 5A and C. We have qualitatively checked that, under the employed culture conditions, endothelial cells indeed express the surface layer of interest (see Fig. 5B). Such endothelialized channels have then been used for a proof-of-principle experiment in which we have shown that, with RBCs suspended directly in the growth medium suitable for HUVEC, the endothelial cells are maintained alive during long (several hours) assays in which we image RBCs flowing and interacting with the channel walls (Fig. 5D). This highly encouraging first step sets the basis for further studies of RBC dynamics in realistic biomimetic microchannels, in which the state and activity of the endothelial surface layer can be biochemically controlled.

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