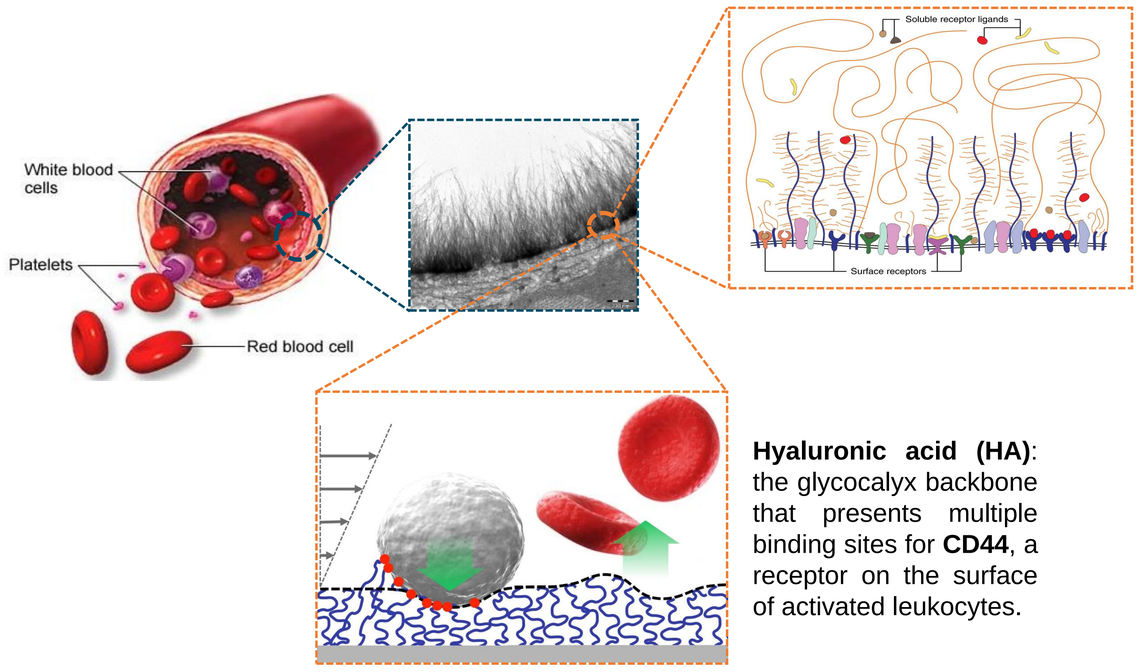
The mechanical modulation of cell adhesion under flow by the endothelial glycocalyx

Elena Murillo Vilella[1], Oksana Kirichuk[1], Lionel Bureau[1], Ralf P. Richter[2], Delphine Débarre[1]

[1] *Université Grenoble Alpes, LIPhy, 38000 Grenoble, France*
[2] *School of Biomedical Sciences, Faculty of Biological Sciences, University of Leeds, Leeds LS2 9JT, United Kingdom*

1. INTRODUCTION: the gate-keeper at the blood vessel walls

The glycocalyx: a soft (10-100Pa), thick (0.1-1µm) and dynamic polymer brush that covers the vessel walls and regulates the attachment of cells.



Hyaluronic acid (HA): the glycocalyx backbone that presents multiple binding sites for **CD44**, a receptor on the surface of activated leukocytes.

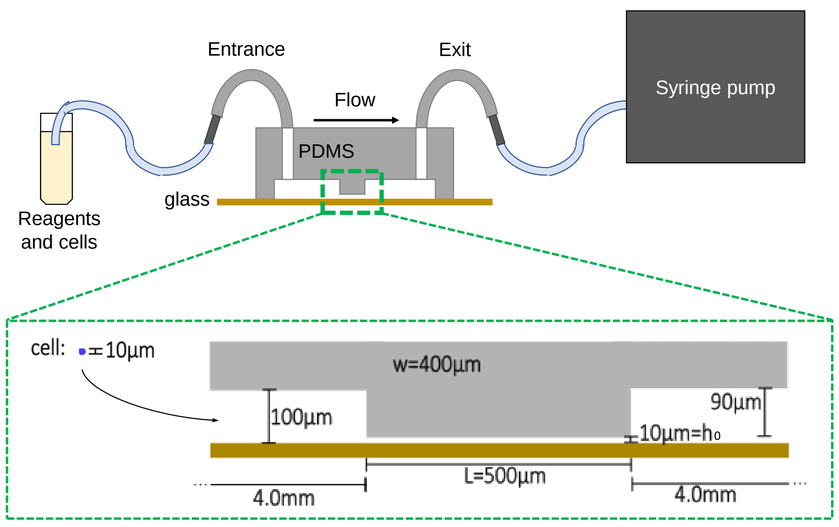
The softness induces two opposed effects

- Repulsive elastohydrodynamic lift due to the deformation of brush and cells under flow.
- Increase of the number of HA-CD44 bonds due to the indentation.

AIM: to determine if overall the softness of the HA brush hinders or promotes the adhesion of cells presenting CD44.

2. DEVELOPMENT of the *in vitro* setup

(a) Fabrication of a microchannel with a constraining thickness



To impose the physiological conditions of the microcapillaries, the microfluidic channel has a thin region (h_0, L, w) with a similar size than cells (~10µm).

To avoid a large deformation under the imposed pressure drops

↓

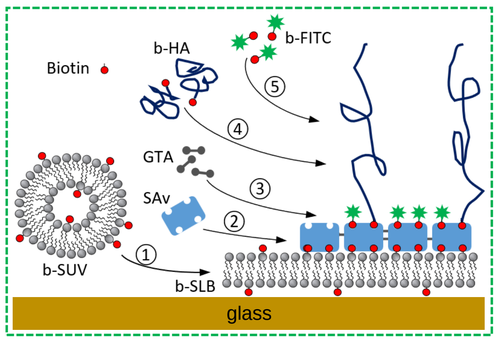
Need to decrease the resistance of the channel: $R_0 = \frac{12\eta L}{w h_0^3}$

↓

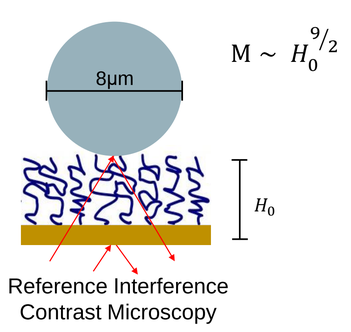
Construction of a channel with two thicknesses, limiting the thin region to a short length.

(b) Functionalization of the surface with a biomimetic glycocalyx

Steps to graft a controlled and well-defined HA brush



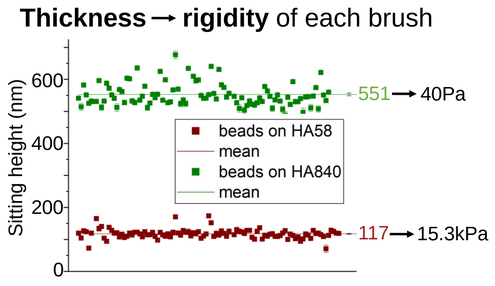
Characterization of the brush rigidity by measuring the thickness *in situ*



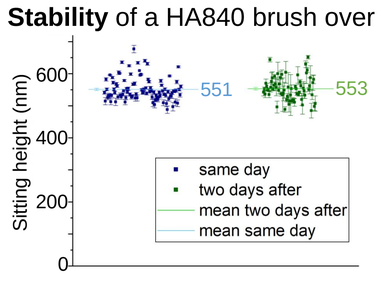
Two different brushes are probed **to focus the study** on just the effect of the softness

- **HA840**: physiologically soft
- **HA58**: 10^3 times more rigid

Thickness → rigidity of each brush

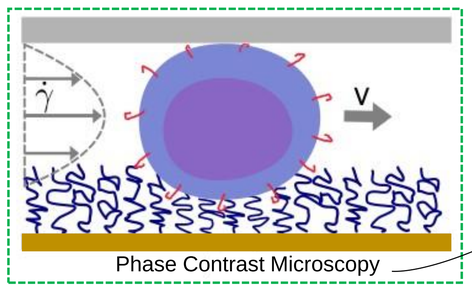


Stability of a HA840 brush over time

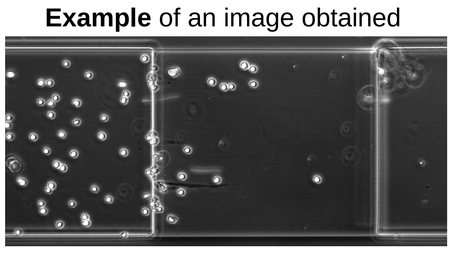


(c) Insertion of CD44+ cells under imposed flows

AKR1 mouse lymphocytes with the transfected CD44 protein are introduced in the functionalized microchannel at controlled flows and recorded.



Example of an image obtained



CD44+ cells upon an HA58 brush flowing at 2µl/min

3. Preliminary RESULTS

Imposed flow (µl/min)	Shear rate (s^{-1})	HA58 (very rigid brush)	HA840 (physiologically soft)
20	50000	No attachment	No attachment
10	25000	No attachment	No attachment
4	10000	No attachment	No attachment
2	5000	Very low, beginning	No attachment
1	2500	Around half or less	Very low, beginning
0.5	1250	Almost all	Low
0.2	500	All cells attaching	Low

(physiological values at 100-1000s^{-1})

In these experiments it was a bit unclear whether the brush had formed well inside the thin region of the channel, so the results call for a confirmation.

4. CONCLUSIONS

- Design, fabrication, and characterization of an *in vitro* system to probe the effect of the softness of the HA brush on the adhesion of CD44+ cells.
- Obtention of preliminary results that show the antiadhesive role of the softness, caused by the deformation of brush and cells under flow.

Related literature

- S. Reitsma, D. W. Slaaf, H. Vink, M. A. M. J. Van Zandvoort, and M. G. A. Oude Egbrink, “The endothelial glycocalyx: composition, functions, and visualization,” *Pflugers Archiv* , vol. 454, no. 3, p. 345, Jun. 2007, doi: 10.1007/S00424-007-0212-8.
- H. S. Davies *et al.*, “An integrated assay to probe endothelial glycocalyx-blood cell interactions under flow in mechanically and biochemically well-defined environments,” *Matrix Biol*, vol. 78–79, pp. 47–59, May 2019, doi: 10.1016/J.MATBIO.2018.12.002.
- H. S. Davies, D. Débarre, N. El Amri, C. Verdier, R. P. Richter, and L. Bureau, “Elastohydrodynamic Lift at a Soft Wall,” *Phys Rev Lett*, vol. 120, no. 19, May 2018, doi: 10.1103/PHYSREVLETT.120.198001.
- G. Coupier, B. Kaoui, T. Podgorski, and C. Misbah, “Noninertial lateral migration of vesicles in bounded Poiseuille flow,” *Physics of Fluids*, vol. 20, no. 11, Nov. 2008, doi: 10.1063/1.3023159/256148.