Soft and sticky: Hyaluronan at the blood vessel wall homes CD44+ beads under flow

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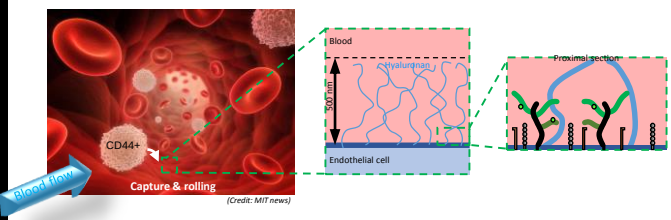
Introduction

How do circulating cells initially attach to the blood vessel wall?



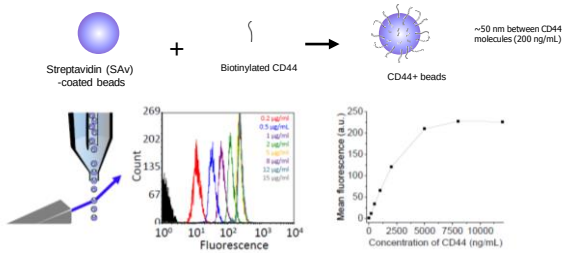
At the blood vessel wall, hyaluronan (HA) polymers provide a soft, deformable layer (the glycocalyx) and present many binding sites for the cell surface receptor CD44.

How do these physical properties of HA influence HA-CD44-mediated cell rolling under flow?

- Pinpointing these factors is extremely complex *in vivo*
- We have developed a unique surface platform to study the rolling behaviours of CD44-coated particles under flow.

Generation of CD44+ cell mimetics

CD44+ bead formation and characterisation by flow cytometry

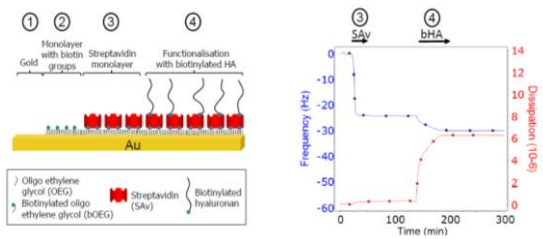


- Beads have 25 µm diameter (lymphocytes are typically 6-14 µm)
- Cell-mimetics with tunable CD44 density.

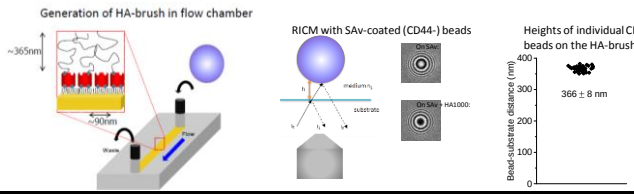
Generation of glycocalyx mimetics with controllable flow

HA-brush formation and monitoring by Quartz-crystal microbalance with dissipation monitoring (QCM-D)

Surfaces were made using HA of ~1000 kDa (contour length ~ 2100 nm).

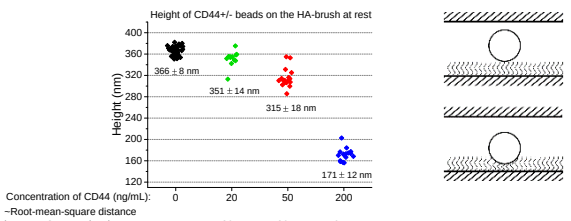


Reflection interference contrast microsopy (RICM) demonstrates that the HA-brush thickness mimics that of the endothelial glycocalyx



CD44+/- beads on the HA-brush at rest

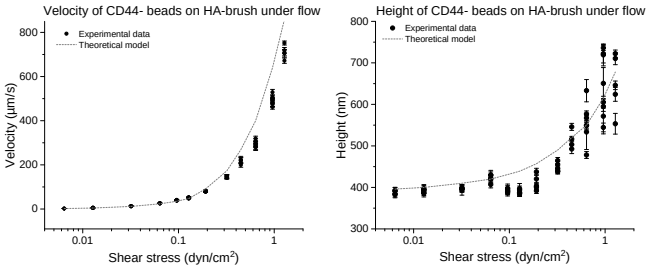
CD44+ beads substantially indent the soft HA brush due to specific interactions at rest



CD44- beads on the HA-brush under flow

Beads that do not interact with the HA-brush show uplift under flow

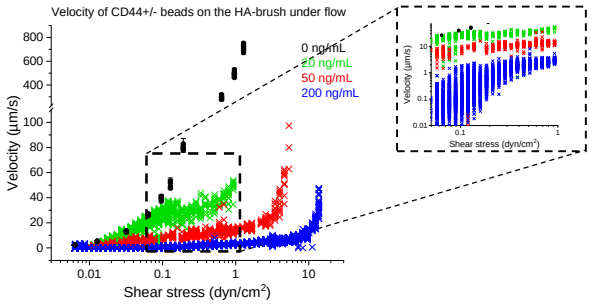
Bead rolling behaviour was examined at various flow-rates, to impose a range of shear stress including that reported in post-capilliary venules (~1-5 dyn/cm^2). Theoretical predictions of velocity[1] and height[2] were computed using an elastic modulus of 200 Pa[3].



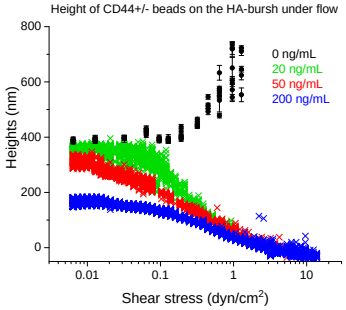
- To our knowledge this is the first experimental evidence of the hydrodynamic uplift of non-interacting particles on a deformable surface.
- This suggests that the deformable nature of the endothelial glycocalyx could contribute to the streaming of RBCs away from the vessel wall.

CD44+ beads on HA-brush under flow

CD44+ beads have substantially reduced velocity on the HA-brush, due to specific interactions



CD44+ beads fully indent the soft HA-brush under flow



- This indentation could enable rolling immune cells to engage with adhesion receptors at the blood vessel wall and facilitate firm adhesion.

Conclusions and perspectives

Non-interacting beads demonstrate the softness of the HA-brush and show the first experimental evidence of 'uplift' under force

CD44+ beads interact with the HA-brush under flow and fully indent it

Going forward:

- HA50 and HA15 brushes will be used to alter the softness of the HA-layer
- Flow assays using CD44+ cell line and primary cells
- Are HA-CD44 interactions catch bonds?

Acknowledgements and References

This work is supported by the UGA-Emergence and PRESTIGE co-funding schemes (09/2015-12/2016) and Centre National D'Études Spatiales (CNES, 01/2017-present). Thanks go to David Jackson, The University of Oxford, for providing bCD44, and to Thomas Combriat (UGA) for the bead-brush schematic diagram.

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