

## Near-wall microfluidics and the glycocalyx in venules

The interface between blood and the vascular endothelium represents one of the most complex, dynamic, and fundamentally important interfaces in mammalian physiology. In particular, this interface represents the first barrier in transcapillary exchange, is a determinant of capillary permeability, is the site of essential steps in the complement and coagulation cascades, and is an important site of free radical interactions. Strategically located at this interface is an glycocalyx surface layer that is regulated by and expressed on capillary endothelial cells. The glycocalyx is a macromolecular carbohydrate-rich extracellular matrix, containing among other things, glycoproteins, proteoglycans, glycosaminoglycans, and adsorbed plasma proteins.

We have shown, using high-resolution, near-wall, intravital fluorescent micro-particle image velocimetry ( $\mu$ -PIV) in the plasma-rich zone of mouse cremaster-muscle venules, that the glycocalyx exerts a significant effect on near-wall microfluidics. From our  $\mu$ -PIV data of the instantaneous translational speeds and radial positions of FITC-labeled microspheres, obtained using dual-flash epi-illumination in an optical section through the median plane of the vessel, we have inferred fluid-particle translational speeds from a detailed three-dimensional analysis of the local fluid dynamics in the vicinity of the vascular endothelium and its glycocalyx. Based on our analysis of  $\mu$ -PIV data in vessels before and after light-dye treatment to degrade the layer, our results are consistent with a strongly exponential rather than linear velocity distribution very near the vessel wall in control vessels. From these distributions, we have been able to provide the first direct estimates of the effective hemodynamically relevant thickness of the layer *in vivo* (see figure 1).

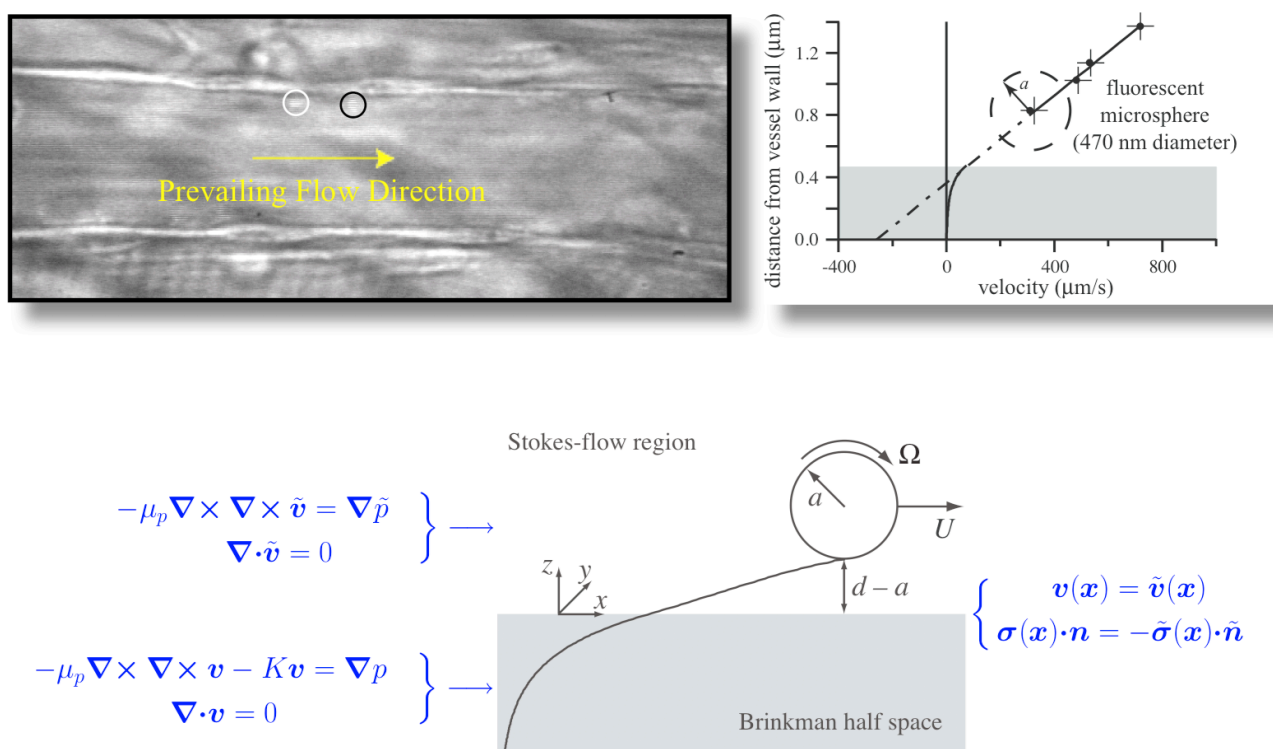


FIGURE 1: Typical bright-field image (top left) showing a dual image of one microsphere ( $0.47 \mu\text{m}$  diameter) in a mouse cremaster muscle venule *in vivo*. The dual images of the microsphere (encircled in white upstream and black downstream) are separated in time by the double flash interval. Fluorescent intravital  $\mu$ -PIV data in the plasma-rich region of a mouse cremaster muscle venule (top right,  $32.8 \mu\text{m}$  diameter) showing measured translational speeds (dot symbols) of sphere centers and predicted translational speeds (plus symbols) of fluid particles if the spheres were not present in the flow. Based on the measured translational speed of a microsphere centered a distance  $h$  from the vessel wall, we determined the speed a fluid particle would have at a distance  $h$  from the vessel wall in the absence of the microsphere by invoking the analysis of the free motion of a neutrally buoyant sphere in a uniform shear field near a Brinkman half-space (bottom). In every vessel we observed, linear extrapolation (dash-dotted line, top right) of the linear fit to the estimated fluid-particle velocity data revealed a negative intercept at the vessel wall. Permeation-induced fluid drag through the glycocalyx is thought to account for the enhanced drag on the portion of the microsphere that is nearest to the glycocalyx interface. If flow through the glycocalyx were neglected, layer thickness is estimated as the distance from the vessel wall where the linear fit extrapolates to zero velocity, whereas for finite values of the hydraulic resistivity,  $K$ , glycocalyx thickness is estimated using predicted velocity profiles in the layer from our analysis of the motion of sphere near a Brinkman half-space.

This represents the first such estimate of its kind. Whereas all other estimates of layer thickness *in vivo* are metrics for the thickness of a steric exclusion zone to macromolecules, our results have allowed estimates beyond those previously possible, because we have directly shown a significant hydrodynamic effect of the layer on near-wall microfluidics. For flow conditions typical of the microcirculation, the extent of plasma flow retardation through the layer that we have estimated would result in near complete attenuation of fluid shear rate and fluid shear stress on the endothelial-cell surface. Consequently, the transmission of mechanical stress from the fluid flow in the lumen to the endothelial-cell cytoskeleton is likely to occur through the transfer of stress from fluid flow through the glycocalyx near its apical end to proteoglycans with transmembrane domains at the base of the layer. In our view, the endothelial-cell lipid bilayer bears very little stress from the prevailing flow in the vessel, as most of this stress is communicated from the glycocalyx directly to the endothelial-cell cytoskeleton. A layer with these properties has significant implications for microvascular physiology. In particular, the direct demonstration of a hemodynamically relevant glycocalyx necessitates the revision of previous concepts of wall shear rate, wall shear stress, leukocyte adhesion, microvascular flow resistance, stress transmission to vascular endothelium, and endothelial mechanotransduction mechanisms.

## REFERENCES

- DAMIANO, E. R., LONG, D. S., EL-KHATIB, F. H. & STACE, T. M. (2004a) On the motion of a sphere in a Stokes flow parallel to a Brinkman half space. *J. Fluid Mech.* **500**, 75–101.
- SMITH, M. L., LONG, D. S., DAMIANO, E. R. & LEY, K. (2003) Near-wall  $\mu$ -PIV reveals a hydrodynamically relevant endothelial surface layer in venules *in vivo*. *Biophys. J.* **85**, 637–645.