Understanding the interplay between nanoparticle properties and interactions with the endothelium using *in vitro* blood vessel models

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Despite considerable investment in the development of nanoparticle drug delivery systems, only a small fraction, as low as 2%, of the administered dose actually reaches its intended target sites[1]. Blood vessels act as both the primary pathway for delivery systems within the body and a challenging barrier to accessing target tissues. Progress in developing *in vitro* blood vessel models is anticipated to facilitate the design and preclinical evaluation of new delivery systems capable of effectively binding to and traversing the blood vessel wall.

The endothelium coats the inner lining of all blood vessels, with the endothelial cell glycocalyx serving as the primary tissue barrier at the blood interface. Comprising sugar macromolecules anchored at the cell membrane via proteins and lipids, the glycocalyx forms an anionic hydrogel-like interface[2]. Initially perceived as a passive surface coating, the endothelial glycocalyx is now recognised as pivotal in regulating substance transport across cell membranes. However, we do not fully appreciate the impact of the glycocalyx on biomaterial interactions at the cell surface.

In this study, we refined the culture conditions of primary human endothelial cells, exposing them to either static conditions or shear stress mimicking blood flow. This optimisation aimed to induce functional endothelial properties, such as cell-cell junctions (VE-cadherin expression), barrier functions (assessed via transwell assays), and the expression of major glycocalyx components including heparan sulphate (HS) and hyaluronan (HA). These engineered blood vessel models were utilised to evaluate the binding and internalisation of nanoparticles (*e.g.* gold and polymer micelles, < 100 nm) as well as polymers (*e.g.* chitosan-based) by endothelial cells through microscopy and flow cytometry. The findings suggest that cationic systems exhibit enhanced binding to the glycocalyx and are more readily internalised compared to anionic systems. Furthermore, we demonstrate functional siRNA intracellular delivery in a polyplex with cationic, linear polymers shown by silencing GFP expression.

We expanded our cell-based analyses to construct *in vitro* cell surface models where either HS or HA was immobilised on gold substrates using thiol chemistry. The quartz crystal microbalance together with these surface-tethered approaches facilitate high-resolution, real-time monitoring of molecular interactions and associated alterations in bound water. It suggests that cationic systems bind HS and HA through a condensation reaction, exhibiting a higher affinity for HS compared to HA. This finding supports a proposed mechanism of transport through the glycocalyx for presentation at the cell membrane.

Together this study adds to our understanding of nanoparticle properties that facilitate binding to and transport through the glycocalyx for intracellular uptake. Future work will expand our approach to consider more diverse nanoparticle physicochemical properties including size, morphology, and rigidity.

References:

[1] Cheng, Y. *ACS* Nano **2020**, 30, 3075-3095.
[2] Fu, L. *Advanced Drug Delivery* Reviews **2022**, 184, 114195.