CONTROLLING CELLULAR TRAFFICKING BY NANOPARTICLE AVIDITY: FROM ENDOCYTOSIS TO TRANSCYTOSIS

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Therapeutic intervention in the central nervous system (CNS) is limited by the presence of the blood brain barrier (BBB) that controls the movement of molecules across the BBB. However, transcytosis mechanism can be hijacked to transport therapeutics to the CNS. Polymersomes, synthetic nanoscopic vesicles, represent a promising drug delivery system. They can accommodate a variety of drugs at high concentration and their outer surface can be decorated Angiopep2 peptide (AP2), which enables transcytosis. However, the mechanism regulating ranscytosis of nanoparticles into the brain is poorly characterised. We hypothesised that the intracellular trafficking of polymersomes decorated with AP2 is dependent on the density of ligands on the nanoparticle surface. To test this hypothesis we produced poly[oligo(ethylene glycol) methyl ether methacrylate]-block poly((diisopropylamino)ethyl methacrylate) (POEGMA-PDPA) pH sensitive polymersomes conjugated with different densities of AP2, and assessed transcytosis using a previously established BEC 3D transwell model3.

We show that polymersomes decorated with AP2 bind LRP1 and are internalised by BEC. The rate of transwell crossing varies according to the densities of AP2 conjugated on the surface. Investigating the transcytosis mechanism, we found that a high AP2 density (60 AP2/polymersome) promotes the compartmentalisation of polymersomes in endocytic organelles positive for Rab5, 7 and 11. Contrarily, a medium AP2 density (25 AP2/polymersome) favours transcytosis through tubular structures. Real time live cell imaging studies and molecular dynamic modelling revealed that depending on the avidity of system, cells undergo endocytic vesiculation or tabulation followed by transcytosis. Polymersomes transcytosis is inhibited by small molecule inhibitors to the SNARE complex or dynamin, and also through depletion of cholesterol at the basolateral membrane, indicating an interdependency between endocytosis and exocytosis.

These results underline how ligand density contributes to the intracellular trafficking of nanoparticles. This work provides new insight into the mechanism of transcytosis through the BBB that be exploited for the design of nanotherapeutic vesicles targeting the CNS.

References

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Figures

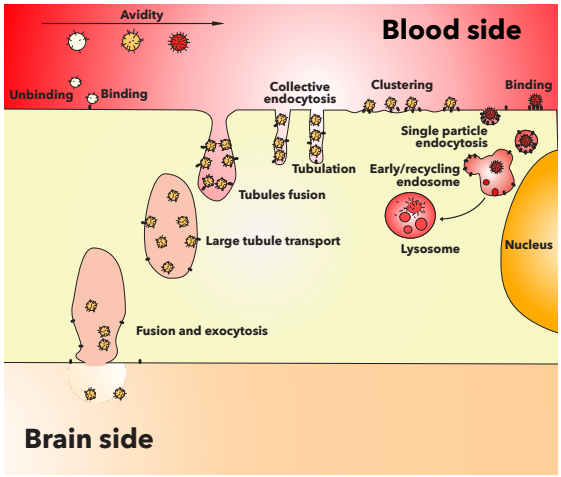

Figure 1. Schematic of the proposed mechanism of transcytosis mediated by nanoparticle avidity.