Real-Time Imaging of Nanoparticle Transcytosis in a Microfluidic Blood–Brain Barrier Model

Yueying Cao[a,b], Guoying Wang[a,b], Xianlin Zheng[a,b], Jun Zhang[a,b], Jia Li[b], Bingyang Shi [a,b], Yiqing Lu[a,b]*

[a] *ARC Centre of Excellence for Nanoscale BioPhotonics (CNBP).*

[b] *Macquarie University, Sydney, 2109, Australia.*

The blood–brain barrier (BBB) plays the pivotal role in maintaining homeostasis of the brain microenvironment, preventing pathogens and toxins from entering the brain [1]. Meanwhile, BBB also impedes the delivery of most therapeutic agents and poses a significant challenge for treating brain diseases [2]. Despite various approaches aiming to enhance BBB penetration have been investigated, the delivery efficiency is generally below 1% [3], suggesting in-depth knowledge of the BBB penetration process is critically required to overcome this barrier. Here we use lanthanide upconversion nanoparticles (UCNP) to visualize the transcytosis process through brain endothelial cells in an in vitro microfluidic BBB model. Incorporating cutting-edge strategies of high doping concentration and inert shell passivation to enhance the luminescence signal [4, 5], single UCNPs are clearly identified under microscopy imaging at 20 frames per second. This allows the movement of the UCNPs to be precisely captured with respect to the endothelial cells after being introduced into the vascular chamber of the BBB model. The integrity of the BBB is continuously monitored using fluorescent dye, showing no compromise during the entire period of the imaging experiment. Thorough quantitative analysis of the UCNP trajectories, we successfully differentiate intracellular from intercellular penetration events based on their dynamic displacement against time for individual UCNP events. This is the first real-time observation of single nanoparticles crossing the BBB, which result will help better understand the underlying mechanisms of BBB penetration and facilitate the development of new nanoparticle-mediated brain therapeutics.

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