Visualising Nanoparticle Delivery in 3D Blood-Brain Barrier and Brain Tumour Organoids

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Introduction: Diffuse intrinsic pontine glioma (DIPG) is a highly aggressive and incurable disease constituting about 10% of all childhood central nervous system tumours[1]. Due to its inaccessible location in the brain, surgery is not feasible and treatment options are limited leading to a dismal prognosis. The intact blood-brain barrier (BBB) in these tumours further adds to treatment inefficiency. Nanoparticle-based therapy is an invaluable approach to improving drug delivery, especially in the brain. Surface functionalisation of nanoparticles, such as lactoferrin, increases the likelihood of tumour cell uptake by binding to receptors that are highly expressed in both the BBB and brain tumours. This has been demonstrated in transwell and 3D cell models[2], whereby lactoferrin-coated nanoparticles were able to efficiently cross the transwell BBB model and penetrate 3D glioblastoma spheroids[3]. Here, we leveraged an *in vitro* 3D BBB model and DIPG organoids to quantitively assess the penetration of these lactoferrin-coated nanoparticles.

Methods: Ultra-small, large pore mesoporous silica nanoparticles (MSNs) were developed using a facile synthesis method and subsequently functionalised with lactoferrin coating (Lf-MSNs)[3] and conjugated with polyethylene glycol (PEG; PEG-Lf-MSNs) to improve stability[4]. To track the uptake and penetration of Lf-MSNs and PEG-Lf-MSNs, confocal microscopy was used to visualise live 3D BBB and DIPG organoids. 3D BBB organoids were established using immortalised endothelial cells, astrocytes, and pericytes (1:1:1). 3D DIPG organoids were grown up to 500 µm in diameter prior to confocal microscopy imaging. A quantitative analysis platform was used to determine the penetration kinetics[5].

Results: Lf-MSNs had enhanced penetration kinetics in live 3D DIPG organoids compared to uncoated MSNs. Interestingly, PEGylation of MSNs further increased penetration in the tumour organoids, with PEG-Lf-MSNs having higher penetration kinetics over the uncoated counterparts. Furthermore, both Lf-MSNs and PEG-Lf-MSNs can cross the *in vitro* 3D BBB model.

Conclusions: Surface functionalisation of MSNs with lactoferrin impacts their penetration in live 3D BBB and DIPG organoids. PEGylation of MSNs further enhances penetration *in situ*. The methods presented here provide insight into nanoparticle design for future *in vivo* prioritisation and development, to improve drug delivery in highly aggressive brain cancers.

References:

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