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Engineering human-relevant glioblastoma microenvironment models for the optimization of advanced drug delivery systems

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The intricate tumor microenvironment (TME) of glioblastoma (GBM) poses significant challenges for personalized therapies due to its histological heterogeneity, stiff extracellular matrix (ECM), and the blood-brain barrier (BBB) [1]. Although nanoparticles (NPs) and cell-based drug delivery systems (DDSs) may address these issues, their success requires rigorous validations through reliable *in vitro* models replicating this complexity [2].

This study presents a three-dimensional GBM model that integrates human TME cells, ECM-mimicking biomaterials, and microfluidic platforms, providing a robust tool to optimize and evaluate innovative DDSs.

GBM spheroids incorporating primary GBM cells (U87), cancer-associated stem cells (GBM-8) and resident brain cells (microglia, astrocytes) were encapsulated in hydrogels of different stiffness to mimic ECM role during tumor progression. This model was employed to evaluate the effect of Bortezomib (BTZ)-loaded NPs on spheroid infiltration. Additionally, NP-loaded microglia were assessed as alternative carriers, given their inherent tropism for GBM.

To verify carrier extravasation across BBB, an *in vitro* brain microvascular network was established using microfluidic platforms (MIMETAS OrganoPlate®), human brain endothelial cells and pericytes. Immunostaining and perfusion assay confirmed network functionality and carrier infiltration.

Tumor spheroids reproduced important GBM features, such as necrotic cores and stem cell niches. BTZ-NPs successfully inhibited spheroid growth in ECM-like hydrogels, with efficacy depending on cellular composition, and reduced drug cytotoxicity on resident cells compared to free BTZ. However, NPs penetration and effectiveness were hampered in stiffer matrices, whereas microglia-based DDS demonstrated superior infiltration and significantly decreased tumor cell viability.

The brain vascular network model featured homogeneous vessels supporting spheroid vascularization. Immunofluorescence staining confirmed the presence of tight junctions, replicating the *in vivo* barrier effect against NPs. Furthermore, targeted extravasation of microglia-based DDSs towards GBM was demonstrated.

This sophisticated human-relevant model leverages biomimetic biomaterials and microfluidics to robustly screen novel GBM therapies. By accurately replicating TME complexity, the system enables the validation of advanced biomimetic carriers, paving the way for their translation to clinical application.

References

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Translocation of LRP1-targeted carbon nanotubes across the blood-brain barrier *in vitro* and *in vivo*

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The blood-brain barrier (BBB) is a major obstacle for drug delivery to the brain, limiting the number of drugs reaching the market to tackle brain disorders [1]. There is increasing research in the use of nanoparticles as transporters to facilitate BBB crossing and effective delivery to the brain. Carbon nanotubes have attractive properties that make them suitable candidates as drug delivery vectors to the brain, such as high aspect ratio and the ability to penetrate biological membranes due to their tubular shape.

However, it is important to increase carbon nanotube specificity in order to achieve a better internalization. Angiopep-2 is a ligand of LRP1 receptor (low-density lipoprotein receptor-relative protein-1), expressed in several tissues including brain capillary endothelium and gliomas [3]. In this work we have investigated the ability of Angiopep-2-functionalised carbon nanotubes to cross the BBB *in vitro* and *in vivo*. The co-culture model was prepared using porcine brain endothelial cells (PBEC) and primary rat astrocytes. Angiopep conjugation significantly increased the BBB transport and brain uptake of carbon nanotubes, as demonstrated by transmission electron microscopy (TEM) and gamma counting. These results suggest the potential of carbon nanotubes as drug delivery vectors to treat diseases such as glioblastoma.

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