Oral comunication

In vitro Human-relevant Glioblastoma Models as the novel frontier of nanomedicine screening

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The highly heterogeneous tumor microenvironment (TME), the stiff extracellular matrix (ECM) and the Blood Brain Barrier (BBB) hinder treatment efficacy against Glioblastoma (GBM). Hence, the preclinical evaluation of novel drug delivery platforms is key in GBM management. Currently, drug screening relies on animal studies or *in vitro* models, which do not fully replicate GBM complexity. To fill this gap, this study aims to develop a human-relevant GBM model to investigate the efficacy of nanoparticles (NPs)-based drug delivery systems.

Multicellular tumor spheroids (MTS) were prepared by mixing different cell types at varying ratios (e.g., tumor cells, microglia, and GBM-Stem Cells) to model GBM composition and embedded in polymeric hydrogels resembling the main properties of GBM ECM. MTS infiltration capacity and viability were assessed on the model following treatment with polyurethane NPs for the controlled release of Bortezomib (BTZ), a proteasome inhibitor. The results confirm that BTZ can reduce tumor proliferation and infiltration in ECM-like gels, with the effect depending on the cellular composition.

To verify NPs extravasation across brain capillaries, a vascular network was inducted into the MTS through a commercial microfluidic platform, using brain capillary endothelial cells. Immunostaining and perfusion assays were performed to analyze microvessels properties. CD31-staining showed the homogeneous presence of endothelial cells forming tight junctions (confirmed by ZO-1 staining). Fluorescent NPs injected in the channels were retained without extravasation, confirming previous *in vivo* observations.

This model represents a prototype for a 3R-compliant replica of GBM microenvironment, combining key cell actors, biomimetic materials, and an *in vitro* brain microvasculature. The promising results suggest the possibility to increase model complexity, e.g., by including pericytes and astrocytes, to provide a reliable tool for nanomedicine screening.