Design of Bioengineered Glioblastoma Microenvironment Models for the validation of innovative nanomedicine

A. Bezze, S. Muccio, G. Ciardelli, C. Mattu

Purpose/Objectives:

The development of effective treatments for aggressive diseases like glioblastoma (GBM) is impeded by the intricate tumour microenvironment (TME). The TME's high histological complexity, stiff extracellular matrix (ECM), and the presence of the blood-brain barrier pose significant challenges for identifying therapeutic targets and hinder drug accumulation. In order to facilitate the development of innovative therapies and drug carriers that can overcame the barriers represented by GBM TME, it is imperative to establish reliable *in vitro* models that faithfully replicate its intricate complexity.

This study aims to create a robust three-dimensional model of GBM for studying the transport dynamics of drug delivery platforms based on polymer nanoparticles (NPs). This model integrates various cell types present in human GBM, biomaterials resembling the tumour ECM, and a microfluidic device to reproduce the vascularization process, ensuring an accurate representation of GBM's structure and composition.

Methodology:

GBM spheroids, incorporating different primary glioblastoma cells (U87/U251), cancer-associated stem cells (GBM-8), microglia (HMC3), and astrocytes (HASTR), are encapsulated in natural or synthetic polymeric hydrogels with mechanical properties akin to the GBM matrix. The model was employed to assess the effect of the administration of polyurethane NPs for the controlled release of Bortezomib (BTZ), a proteasome inhibitor, on the infiltration capacity and viability of the GBM spheroids.

To verify NPs extravasation across brain capillaries, vascular network was established by introducing GBM spheroids into a commercially available microfluidic platform featuring two lateral perfusion channels coated with human brain endothelial cells. Angiogenic sprouting was then induced to facilitate the vascularization of the spheroid.

Results:

The GBM spheroids can replicate some important features of the tumour, such as the presence of the necrotic core and the formation of the different stem cells niches (Fig.1A). The viability results on GBM spheroids after the administration on GBM spheroids showed that the treatment efficacy increases over time and with nanoparticles concentration. Similarly, BTZ and BTZ-NPs can reduce tumour proliferation and infiltration in ECM-like gels, with efficacy dependent on cellular composition. While NPs-mediated treatment exhibits lower efficacy compared to the free BTZ treatment, it minimizes cytotoxicity on non-tumour cells within the TME.

The microfluidic-based vascular network model includes dense and homogeneous vessels forming well branched sprouts and supports spheroid vascularization (Fig. 1B). Furthermore, the immunofluorescence staining confirms the presence of endothelial cells forming tight junctions (Fig. 1C), replicating the barrier effect against NPs observed *in vivo*.

Moreover, the developed model enables the replication and investigation of microglia extravasation mechanisms and tumour homing within the GBM TME (Fig.1D), indicating its potential as a validation platform for the advancement of cell-mediated transport systems.

Conclusion/Significance:

This innovative model represents a significant stride in replicating the human GBM TME by combining biomaterials and microfluidics. It offers a valuable tool for the preliminary validation of nanomedicines,

facilitating the exploration of new materials and delivery mechanisms, such as cell-mediated drug delivery, to overcome the challenges posed by the TME in glioblastoma treatment.

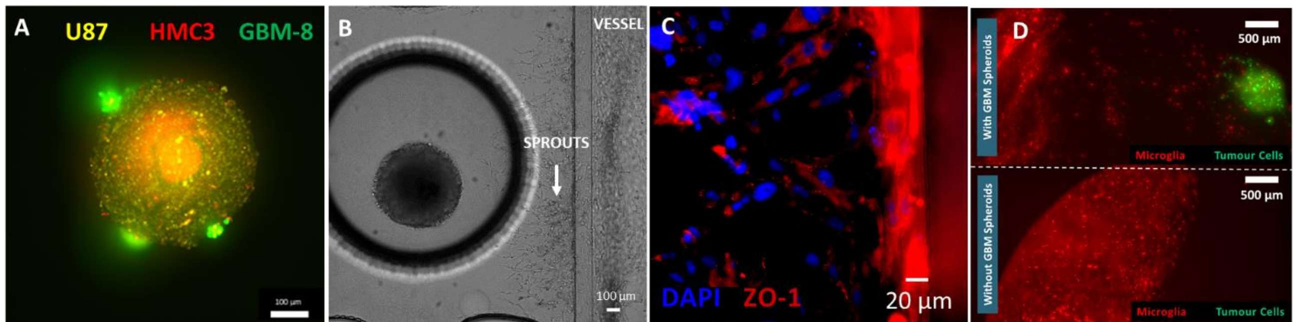

Figure 1: A) Fluorescence image of the distribution of GBM cells (U87), Cancer Stem Cells (GBM-8) and microglia (HMC3) within the spheroids. B) Brain endothelial vessel sprouting in the microfluidic platform towards GBM spheroids through ECM-like hydrogels . C) Thigh junction-associated marker distribution in the artificial vessel. D) Fluorescence imaging of HMC3 cells in collagen hydrogels with or without TM spheroids.