**pH-responsive polymersomes: precision drug co-delivery against glioblastoma**

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**Background:** Glioblastoma (GBM) is the most aggressive brain tumor with poor prognosis and limited treatment options, largely due to the blood-brain barrier and the tumor's invasive nature and recurrence. pH-responsive polymersomes, which can co-deliver multiple agents and respond to the acidic tumor environment, show promise for controlled drug release, improving efficacy and reducing side effects. Additionally, a potentially injectable chitosan hydrogel enhances the system's anti-tumoral properties and enables local, sustained drug release, improving the potential for clinical applications.

**Methods:** This study developed and evaluated pH-responsive polymersomes for GBM treatment via sustained, controlled drug co-delivery. The polymersomes were prepared from pH-responsive block copolymers, engineered to self-assemble in stable vesicles under physiological conditions but disassemble in acidic tumor environments. In vitro, the pH-sensitive drug release profile was assessed in a range of GBM-relevant pHs. Cytotoxicity assays and cellular uptake studies were performed in 3D GBM patient-derived organoids to evaluate the safety and therapeutic efficacy of the polymersomes. For in vivo validation, the polymersomes were embedded in a thermoresponsive chitosan hydrogel for sustained local delivery and their safety and therapeutic efficacy were tested in a GL261 tumor resection mouse model.

**Results:** The polymersomes exhibited a pH-sensitive release profile, with a 2-fold drug release triggered at acidic pH levels typical of the tumor microenvironment in GBM. The in vitro cytotoxicity studies demonstrated the empty nanocarriers had no effects in cell viability, rendering them safe for their use in GBM. Their therapeutic efficacy was confirmed against patient-derived GBM 3D organoids, displaying significant levels of tumor cell death. Their efficacy was improved further via the co-delivery of drugs due to synergistic effects. The cellular uptake studies confirmed the efficient cell internalization of the polymersomes and successful release of their therapeutic payload in the same model. Finally, in vivo studies using a GL261 tumour resection mouse model validated the safety and effectiveness of the polymersomes, which were loaded in a thermoresponsive chitosan hydrogel for local delivery and potential injectability. The final nanocomposite system demonstrated prolonged drug release, reduced tumor recurrence, and improved survival rates compared to controls.

**Conclusions:** This study highlights the suitability of pH-responsive polymersomes as an advanced drug co-delivery system for the treatment of GBM. The ability of these polymersomes to selectively deliver multiple therapeutic agents and sustain their release through a potentially injectable chitosan hydrogel platform shows significant potential for improving treatment outcomes against recurrent GBM.