

Layer-by-Layer Nanoparticles for Targeted Delivery and Treatment of Glioblastoma

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Glioblastoma (GBM) is the most common and lethal malignant primary brain tumor in adults, characterized by rapid growth and diffuse infiltration throughout the brain. Despite an aggressive multimodal standard of care – including surgical resection, radiotherapy, and chemotherapy – nearly all patients experience recurrence, with a median survival of only 12-15 months post-diagnosis. Conventional chemotherapy is ineffective as most drugs cannot cross the blood-brain barrier (BBB) at therapeutic levels without inducing systemic toxicity. Convection-enhanced delivery (CED) offers a promising alternative by directly infusing therapies into the brain, achieving high local concentrations while reducing systemic side effects. However, the therapeutic efficacy of CED is limited by rapid drug clearance from the brain and non-specific uptake of therapies by surrounding healthy cells.

Nanoparticle (NP) encapsulation offers a promising strategy to extend the retention of locally delivered therapies in the brain and selectively target GBM cells. However, effective NP distribution is limited by the brain's narrow, tortuous pores and harsh ionic environment. Recent studies have highlighted the need for high colloidal stability and minimal adhesion to extracellular matrix (ECM) components to enable penetration through dense brain tissue. However, conventional surface modifications that reduce ECM adhesion can also limit cancer cell uptake. The layer-by-layer (LbL) assembly method – where oppositely charged polyelectrolytes are sequentially adsorbed onto colloidal substrates – provides a modular platform to rapidly test a diverse range of distinct NP surface chemistries. These charge-dense, hydrated films have been demonstrated to promote antifouling properties while enabling multivalent interactions with cell membrane receptors for enhanced uptake.

This thesis applies LbL functionalization to investigate how NP surface chemistries impact the therapeutic efficacy of chemotherapy delivered via CED. The first part of this thesis evaluates a diverse panel of LbL surface chemistries applied to anionic liposomes, a clinically relevant NP core tested in CED clinical trials. Screening these NPs for cellular association with a broad range of patient-derived GBM and healthy neuronal cells across various media and growth formats reveals trends in cellular interactions and identifies hyaluronic acid (HA) and poly-L-glutamic acid (PLE) as consistently high-affinity GBM-selective coatings. To ensure colloidal stability in physiological conditions, we introduce a technique for incorporating polymer blends into outer layers incorporating a PLE-polyethylene glycol (PEG) block copolymer, achieving high stability without compromising selective GBM interactions.

Next, we assess the spatial distribution, retention, and cellular uptake of LbL NPs *in vivo* in both healthy and GBM tumor-bearing mice. Functionalizing the liposome cores with gadolinium allows real-time MRI imaging to quantify distribution volumes and retention following CED. Our findings show that LbL functionalization – particularly with PLE as the outer surface chemistry – promotes broad distribution across healthy and tumor-bearing brain tissue, with a prolonged

retention half-life of approximately 13 days, compared to less than 4 hours for an unencapsulated small molecule. Using a custom multicolor flow cytometry panel and tissue dissociation protocol, we analyze the cellular composition of NP-associated cells, revealing enhanced tumor cell uptake and selectivity, as well as distinct cellular fates for HA- and PLE-coated NPs.

Finally, we leverage the enhanced distribution, retention, and GBM-cell targeting of LbL NPs featuring PLE surface chemistry in a therapeutic application. Covalent conjugation of the potent microtubule inhibitor monomethyl auristatin-F (MMAF) to lipid headgroups on the NP surface resulted in striking potency against a range of patient-derived GBM cell lines that exceeded free MMAF and antibody-drug conjugate (ADC) of MMAF currently under clinical investigation. *In vivo*, a single CED infusion of LbL-functionalized MMAF NPs in orthotopic GBM-bearing mice significantly prolonged survival by improving the tumor exposure of NPs and the MMAF payload. This treatment also achieved a high degree of selective apoptotic activity against GBM cells, supporting the potential of this platform to enhance local therapy by maximizing tumor drug exposure with minimal systemic toxicity.