**Drug Encapsulated Aerosolized Microspheres as a Biodegradable, Intelligent Glioma Therapy**

J. Alaina Floyd¹, Anna Galperin², Rohan Ramakrishna³, Robert Rostomily³, Buddy D. Ratner¹,²

¹University of Washington Dept of Chemical Engineering, ²UW Dept of Bioengineering, ³UW Dept of Neurological Surgery

---

**Statement of Purpose:** In 2013, the American Cancer Society estimated that over 20,000 malignant tumors of the brain or spinal cord will be diagnosed in the United States. Over half of these tumors will prove fatal. Ultimately, despite maximal therapies of tumor resection, chemotherapy, and radiation, median survival remains low at approximately one year due to the inability of a full tumor resection, allowing regrowth and eventual tumor drug resistance. Herein, we present the development of a topical, slow release, multi-drug delivery system applied post-surgically with the potential to significantly increase patient life expectancy. This system consists of poly(lactic acid) (PLA), poly(lactic-co-glycolic acid) (PLGA), and poly(ε-caprolactone) (PCL) drug encapsulated microspheres suspended in a degradable poly(N-isopropylacrylamide) (PNIPAM) solution. At room temperature, PNIPAM solution suspends drug encapsulated microspheres that can be “sprayed on” the post-surgical site. The physiological temperature of the brain causes the thermoresponsive PNIPAM to solidify and form an adherent gel layer with entrapped microspheres (Fig. 1), providing intimate contact with remaining tumor cells. Over time, PNIPAM degrades along with PLGA, PLA, and then PCL microspheres, releasing multiple chemotherapeutics at different rates directly to the tumor remnants to inhibit cancerous regrowth. This work focuses on encapsulation, characterization, and release of rhodamine B (RB), a hydrophilic model drug, and gefitinib, a hydrophobic chemotherapeutic, from PLGA in addition to the encapsulation and characterization of the chemotherapeutic topotecan in PCL and the antibody, immunoglobulin G (IgG), in PLA.

**Methods:** Drug loaded PLGA, PLA, and PCL microspheres were produced by a water/oil/water double emulsion, solvent evaporation technique. Briefly, the solution of polymer in dichloromethane was homogenized with an aqueous solution of the drug. Then, an aliquot of this water in oil solution was added to a poly(vinyl alcohol) aqueous solution and homogenized. This solution remained overnight for solvent evaporation, was stirred, centrifuged, and filtered for microsphere collection. A drug release study was performed at 37 °C with gentle shaking. At specified time points, the samples were centrifuged, and filtered for microsphere collection. A drug release study was performed at 37 °C with gentle shaking. At specified time points, the samples were centrifuged, and filtered for microsphere collection. A drug release study was performed at 37 °C with gentle shaking. At specified time points, the samples were centrifuged, and filtered for microsphere collection. A drug release study was performed at 37 °C with gentle shaking. At specified time points, the samples were centrifuged, and filtered for microsphere collection. A drug release study was performed at 37 °C with gentle shaking. At specified time points, the samples were centrifuged, and filtered for microsphere collection. A drug release study was performed at 37 °C with gentle shaking. At specified time points, the samples were centrifuged, and filtered for microsphere collection. A drug release study was performed at 37 °C with gentle shaking. At specified time points, the samples were centrifuged, and filtered for microsphere collection. A drug release study was performed at 37 °C with gentle shaking. At specified time points, the samples were centrifuged, and filtered for microsphere collection. A drug release study was performed at 37 °C with gentle shaking. At specified time points, the samples were centrifuged, and filtered for microsphere collection. A drug release study was performed at 37 °C with gentle shaking. At specified time points, the samples were centrifuged, and filtered for microsphere collection.

**Results:** Emulsions were used to formulate the polymeric microsphere with particle size dependent on the stirring speed used to form the emulsion. For example, PLA microspheres formed at a 1200 and 2800 rpm emulsion speed resulted in average sphere diameters of 28 ± 12 µm and 11 ± 8 µm, respectively. The microspheres were characterized by SEM and exhibited a smooth and nonporous morphology for RB, gefitinib, topotecan, and IgG encapsulation. The *in vitro* release profile of RB from PLGA showed two distinct regimes of controlled release (Fig 2). Until day 6, there was a moderate, continuous release attributed to diffusion. After day 6, there was a significant increase in the release curve slope, indicating polymer degradation that resulted in a larger release rate before leveling off at day 20. Smaller spheres released faster during the diffusion period due to a higher surface area to volume ratio, showing release profile tunability. The *in vitro* release through PNIPAM (15 mg PLGA/1 mL PNIPAM) showed a similar tendency, with a delay of 2-4 days in release due to the PNIPAM barrier. Gefitinib demonstrated a more prolonged release from PLGA. The diffusion period lasted for 20 days with the bulk degradation occurring from day 20-40 before leveling off. This showed the tunability of the system dependent on molecule type in addition to microsphere size. Gefitinib release from PLGA microspheres in PNIPAM was delayed for 4-6 days. Initial *in vitro* studies for drug bioactivity demonstrated that gefitinib inhibits cell growth after a 20 day release period from PLGA microspheres.

**Conclusions:** Drug loaded PLGA, PLA, and PCL microspheres were formulated using the emulsion technique. Two modes of release, diffusion and degradation, were seen for both RB and gefitinib encapsulated PLGA microspheres. The PNIPAM carrier did not impact release beyond a 2-4 day delay. Current and future studies are focused on the release of topotecan and IgG from PCL and PLA, respectively, evaluating the bioactivity of topotecan against C6 glioma cells *in vitro*, and the encapsulation of an antibody chemotherapeutic in PLA. A pilot animal study will be conducted to evaluate the effect of the delivery system on mice and the diffusion of the chemotherapeutics through the brain.

**Funding:** NSF Graduate Research Fellowship under Grant No. DGE-0718124 and UWEB21