Formation and Characterization of Drug Encapsulated Polymeric Microspheres for Localized Brain Tumor Therapy

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Statement of Purpose: In 2010, the American Cancer Society estimates that 22,070 malignant tumors of the brain or spinal cord will be diagnosed in the United States. Approximately 13,140 (59.5%) of these tumors will prove fatal. Current treatments encompass a variety of methods from surgical excision to radiation therapy and chemotherapy. The tumors' ability to grow back and become drug resistant necessitates a combination of treatments to extend a patient's lifespan. One such combination is surgical removal followed by the application of Gliadel® wafers (Eisai Inc.) to the post-surgical site. These wafers degrade over a period of 2-3 weeks, releasing chemotherapeutics to remaining tumor cells locally. However, the wafers have limited surface contact with the brain tissue and only release a single drug against which a tumor can develop resistance. If a topical, slow release, multi-drug delivery system were developed and applied post-surgically, it is possible that the patient's life expectancy would increase. With this goal in mind, we propose a system based on poly(lactic acid) (PLA), poly(lactic-*co*-glycolic acid) (PLGA), and poly(ε-caprolactone) (PCL) drug encapsulated microspheres suspended in a biodegradable poly(*N*-isopropylacrylamide (PNIPAM) matrix developed in our group. At room temperature, PNIPAM is capable of suspending drug encapsulated microspheres that can be "sprayed on" the post-surgical site. The 37°C temperature of this site would pass PNIPAM through its lower critical solution temperature, causing the polymer matrix to solidify on the surface of the brain, providing intimate contact with the remaining tumor cells. Over time, PLGA, PLA, and then PCL would degrade, releasing multiple chemotherapeutics at different rates directly to the tumor remnants to inhibit cancerous re-growth. The work presented here focuses on forming and characterizing "blank" and encapsulated microspheres of PLA and PCL using light, fluorescent, and scanning electron microscopy. Rhodamine B is used as a model chemotherapeutic drug.

Methods: PLA and PCL microspheres were produced by water/oil/water, solvent evaporation. Briefly, 1.75 g of the polymer was dissolved in 35 mL of dichloromethane (O) and 0.5 g of poly(vinyl alcohol) was dissolved in 50 mL of deionized water (W2). The O solution was homogenized with 1.5 mL deionized water (W1) at 6,000 rpm for two minutes using an Arrow 6000 electric stirrer. Then, 7 mL of this solution was added to the W2 solution and homogenized for one minute at a given speed. The W1/O/W2 solution sat overnight and then stirred for two hours for solvent evaporation. The solution was centrifuged at 3,000 g, 4°C, and 15 minutes with three water rinse cycles before being passed through two Whatman No. 4 filters. Encapsulated spheres were made by adding rhodamine B to the W1 phase at 1 mg/mL. Microsphere shape, size, and fluorescence were determined using a Nikon E800 Upright Microscope. Microsphere morphology was determined using a FEI Sirion XL30 scanning electron microscope.

Results: A five blade circular impeller was used to form PLA and PCL microspheres at two different speeds. A smooth, nonporous microsphere of approximately 50 µm in diameter was the goal for initial experiments. PLA sphere formation was subjected to the two speeds of 2800 and 3560 rpm. The slower speed resulted in larger spheres up to 120 µm in diameter and below and the faster speed resulted in smaller spheres less than 70 µm in diameter. Utilizing scanning electron microscopy (SEM), surface morphology was determined to be smooth and nonporous. Rhodamine B, a fluorescent dye, was encapsulated at the same speeds, resulting in sphere diameters of 80 µm and below for 2800 rpm and 65 µm and below for 3560 rpm. The dye was present when viewed with fluorescent microscopy (Fig. 1) and the sphere morphology remained unaffected by the dye when characterized by SEM.

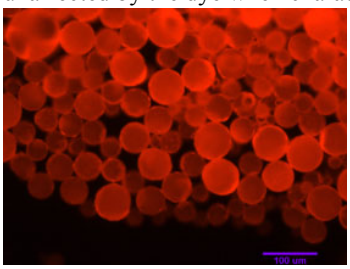

Figure 1 Florescent imaging of rhodamine B encapsulated PLA microspheres formed at 2800 rpm (scale bar 100 µm)

PCL sphere formation was subjected to the two speeds of 2800 and 3240 rpm. The faster speed resulted in spheres 95 µm and below in diameter while the slower speed led to spheres 140 µm and below. When viewed with SEM, the majority of the surfaces were smooth, non-porous and spherical. Rhodamine B encapsulation was then tested, with similar sizes resulting: 140 µm and below for 2800 rpm and 80 µm and below for 3240 rpm. When viewed with fluorescent microscopy, spheres were successfully labeled with rhodamine B. SEM showed that PCL microspheres were also unaffected by the dye, remaining smooth and spherical. Ongoing studies are focused on particle size distributions and encapsulation efficiency of rhodamine B by PLA and PCL.

Conclusions: Both PLA and PCL microspheres of a smooth, spherical nature have been obtained by the methods discussed above. The speed setting has an effect on sphere formation, with the faster speeds resulting in smaller spheres. Successful retention of rhodamine B by PLA and PCL has also been shown, leading to future work to determine the drug release profile *in vitro* along with how changes in the particle size distribution can affect the drug release profile.

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