DEVELOPMENT OF A NOVEL TARGETED MICROPARTICLE DRUG DELIVERY SYSTEM FOR CANCER MEDICATION

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Statement of Purpose: In recent years, a major challenge of the pharmaceutical community has been the development of an effective and less invasive way to target and treat tumors. Half of all men and one third of all women in the United States will develop cancer during their lifetimes[1]. This project proposes a microparticle system for the targeted treatment of cancerous cells. The microparticle is comprised of a polymer shell—poly(D,L lactic-co-glycolide acid) (PLGA)—which will be coated with a covalently linked targeting mechanism for attaching the particles to cancerous cells via an antibody linkage. The targeting method of interest uses a biotin-avidin linkage combined with an antibody for specific targeting. The biotin will be attached to the immediate outer layer of the polymer shell, avidin will readily bind to the biotin, and an antibody will be attached to the avidin. The antibody of choice will be targeted to an upregulated extracellular feature of cancerous cells. The linkage will allow for the drug to be released in areas only containing cancerous cells, leaving healthy cells alone. The focus of this study is to characterize the size, morphology, encapsulation, and release efficiency of the particles using, at first, a model drug, and then a cancer drug.

Methods: Microparticles were originally synthesized using a double emulsion (water in oil in water) process with 5 wt% PVA as the surfactant and ethyl alcohol as the oil phase. Particles were made using a model drug, Bovine Serum Albumin (BSA). Encapsulation efficiency and release testing were then conducted *in vitro*. Attachment of the biotin to the PLGA was accomplished through the photoactivation of TFPA-PEG$_3$-Biotin. Encapsulation efficiency was examined by dissolving dried particles in a water/DCM mixture. Samples of the water phase were taken and tested using a BCA assay kit. Release studies were done at body temperature at sink conditions in phosphate buffer solution for 28 days. At set intervals, 0.5 mL samples were taken and the solution was replenished with fresh PBS after each sample. A BCA assay kit was also used to test the release samples. Once studies with BSA were complete, pacilitaxol was used as the encapsulated drug. Pacilitaxol acts as a mitotic inhibitor. The protocol was altered slightly when Pacilitaxol was used instead of BSA. A similar particle preparation method was used. Release and encapsulation studies will be preformed *in vitro* and analyzed through HPLC testing.

Results: A biotin quantification kit was used to confirm the attachment of TFPA-PEG$_3$- Biotin to the PLGA surface. Florescent microscope (Leica DM 2000) pictures were taken to confirm the presence of biotin on the outer surface of the particles (Figure 1). Size and polydispersity of the particles were examined using a Brookhaven 90 Plus particle size analyzer. SEM and TEM pictures were taken to confirm size and morphology of both PLGA and biotinylated PLGA particles (Figure 2).

Figure 1. 488-Streptavidin Dylight tag to confirm biotinylation on PLGA surface.

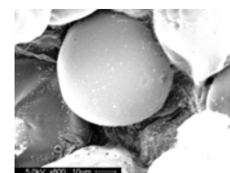

Figure 2. SEM image of biotinylated PLGA particles encapsulating BSA.

PLGA particles were found to be on average 7,800 nanometers, releasing about 50% of the encapsulated BSA over a 28 day period. Encapsulation efficiency gained was 4%. Biotinylated PLGA particles were found to be on average 23,000 nanometers, releasing about 80% of the encapsulated BSA encapsulated over a 28 day period (Figure 3). Encapsulation efficiency for biotinylated particles was 4-5%. In additional studies, the BSA was replaced with a cancer drug, pacilitaxol. PLGA particles encapsulating pacilitaxol were found to be of an average of 2,800 nanometers in diameter. Biotinylated PLGA particles encapsulating pacilitaxol were found to be an average of 20,000 nanometers in diameter.

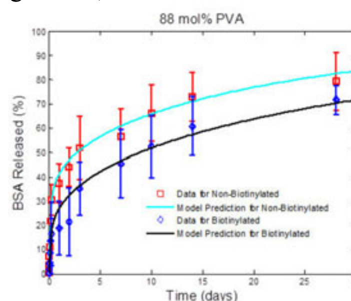

Figure 3: Release of BSA from PLGA and biotinylated PLGA particles.

Conclusions: Microparticles were successfully created using a W-O-W process and demonstrated a controlled release of model drug over a 28 day period. Biotin was successfully attached to the outer surface of the particles, confirmed through fluorescent tagging. Studies showed the attachment of biotin to the outer surface had little to no effect on encapsulation, degradation, or drug release. Microparticles coated with biotin exhibited a controlled release with minimal burst phase and are therefore believed to be effective for anti-cancer therapeutics. Trials using pacilitaxol as a cancer drug are currently being conducted. After this is complete, avidin and an antibody will be attached and trials using cancerous cells will be conducted.

References: *Cancer Basics*. 2012 [cited 2012; Available from:http://www.cancer.org/Cancer/CancerBasics/index.

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