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

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Statement of Purpose: The objective of targeted drug delivery is to improve the efficacy of a given medication. By effectively directing a drug to its intended location, patients can receive smaller dosages with improved results. This project proposes a microparticle design that can be applied for a number of different drugs delivered to specific targeted sites. Cancer cells were studied for this project because of the availability of cell-surface targets and the future benefits associated with improved drug delivery for this group of diseases. The microparticle used is comprised of a polymer shell—poly(D,L lactic-co-glycolide acid) (PLGA) that encapsulates the drug that is to be delivered. The targeting structure is then attached to the outer layer of the microparticle by utilizing an avidin-biotin linkage. The PLGA microparticle's external surface is biotinylated; separately, avidin is linked to an antibody that is specifically targeted to the delivery site. The targeting mechanism is completed by linking the biotinylated microparticle to the avidin-linked antibody. HER2+ breast cancer cells (SKBR3) were targeted with an anti-HER2 antibody to deliver HSA-paclitaxel, a drug acting as a mitotic inhibitor to decrease cell viability of SKBR3. These initial results provided a proof of concept of the targeting, while current studies are focused on improving the size, encapsulation, and release efficiency of the HER2 design to make it viable for in vivo experiments.

Methods: Microparticles were synthesized using a double emulsion (water in oil in water) process where the aqueous phase, HSA-paclitaxel in water, was added to the organic phase, biotinylated-PLGA in ethyl acetate. This primary emulsion was then added to the second aqueous phase, PVA, followed by the addition of the avidn-antibody targeting complex. 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 and evaluating samples of the water phase using HPLC. 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. HPLC was used to test the release of the samples. Once drug release and encapsulation were understood through HPLC results, cell viability tests were conducted using an MTT Assay with various concentrations of HSA-paclitaxel to determine ideal dosage. Particle morphology and size were also measured using a scanning electron microscope (SEM). The targeting mechanism was validated by incubating the SKBR3 cells in the presence of the microparticle, washing the sample to remove unattached particles, and seeing the surface of the cells coated with the PLGA-biotin-avidin-antibody complex

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. 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 1).

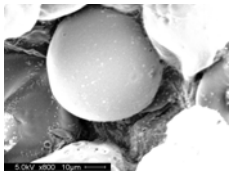

Figure 1. SEM image of biotinylated PLGA particles after encapsulation of drug.

PLGA particles loaded with HSA-paclitaxel and the biotin/avidin linkage were found to be on average 4,100 nanometers in diameter, releasing about 70% of the encapsulated drug over a 28 day period with 42% released in a burst phase occurring at the start of the trial. The microparticle's attachment to the SKBR3 cells were observed under a microscope to validate the targeting mechanism's functionality (Figure 2).

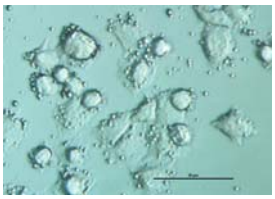

Figure 2. Microscopic image showing attachment of microparticles to SKBR3 cells.

MTT Assay experiments showed a decrease in cell viability from treatments with PLGA to PLGA loaded with HSA/paclitaxel to PLGA loaded with HAS/paclitaxel and the targeting mechanism (Table 1).

Treatment Type (2.5µL)	%Viable
PLGA no drug	85±21
PLGA/HSA/Paclitaxel	68±10
PLGA/Biotin/Avidin/ Antibody/HSA/Paclitaxel	59±3

Table 1. Cell viability results

Conclusions: Microparticles were successfully created using a W-O-W process and demonstrated a controlled release of HSA/Paclitaxel over a 28 day period. Biotin was successfully attached to the outer surface of the particles and the functionality of the targeting mechanism was confirmed with their attachment to the SKBR3 cells. The decrease in cell viability by adding the drug and targeting mechanism prove the benefits of the microparticle/targeting complex. Future studies will examine how to decrease the microparticle size and decrease the burst phase of drug being released. In parallel, a different drug/targeting combination will be selected to further validate the functionality of the design.