Paclitaxel Loaded Polycaprolactone Particles for Treating Endometrial Cancer

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Statement of Purpose: Endometrial cancer (EC) is the sixth most common cancer in women worldwide and is the most common cancer of female reproductive organs.[1] From 2007-2016 there has been a global rise in incidence of EC of 1.3 %.[2] Typically EC is treated with surgery, but at later stages surgery is less effective requiring chemotherapy and/or radiation therapy. These treatment options are less effective than surgery and there are very few FDA approved chemotherapies for EC. The minuscule amount of effective treatment options as well as an increase in incidence shows the need for creating improved treatments for EC. There has been a shift toward using micro- and nano-sized particles as drug delivery systems for chemotherapies due to their ability to decrease side effects of the treatment and increase bioavailability, circulation time, and accumulation of the drug in the tumor. The particles are typically biodegradable polymers and/or lipids. Paclitaxel (PTX) loaded polycaprolactone (PCL) particles were chosen because PTX successfully treats other female cancers and PCL degrades by hydrolysis.[3] We investigated the effects of size separating particles and subsequent cell viability, as well as comparing *in vitro* efficacy in PTX sensitive and PTX resistant cells.

Methods: PTX loaded PCL particles were synthesized using the double emulsion solvent evaporation method (w/o/w). A solution of PTX, PCL, and dichloromethane was used as the oil phase and sonicated for 75s with a 2.5% (w/v) polyvinyl alcohol and DI water. After sonication, they were stirred to allow the DCM to evaporate. The particles then went through sequential centrifugation to separate based on size. When centrifuged at 100rcf the pellet was discarded as unreacted polymer and pellets formed at 200rcf contained particles too large to be useful for *in vitro* analyses. Particles collected at 1000rcf and 3000rcf were washed three times at their respective speeds, resuspended in DI water, frozen, and then lyophilized for 24 hrs. Blank (control) and rhodamine B (RHO) loaded particles were also produced with this method. Particle shape and surface morphology were determined using scanning electron microscopy (SEM). The average diameters were determined using ImageJ. PTX and RHO loading and encapsulation efficiency was determined with high performance liquid chromatography with an ultraviolet detector (HPLC-UV). *In vitro* release was assessed via dialysis for the RHO loaded particles where the particles where mixed with DI water and inserted into dialysis tubing and submerged in a 1% tween-PBS (v/v) release media. Due to low solubility of PTX in water, PTX particles were dissolved in PBS and an octanol layer allowed the PTX to collect in the organic phase. The *in vitro* release was done in an incubated shaker kept at 37˚C and 300 rpm. Two endometrial cancer cell lines, Ishikawa H (PTX resistant) and KLE (PTX sensitive), where investigated for cell viability following PTX-PCL exposure and cellular uptake following RHO-PCL exposure.

Results: With sequential centrifugation, two distinct size distributions of particles were collected. At 1000rcf, the average diameter size across all three particle types was 1.45±0.56 µm. At 3000rcf, the average particle diameter for all particle types was 0.87±.36 µm (Figure 1). The SEM

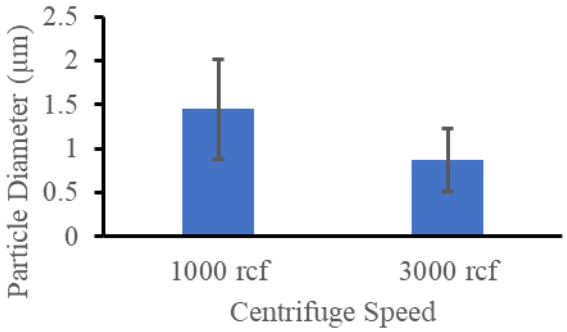

Figure 1:Average particle diameters for all three particle types at 1000rcf and 3000rcf. Error bars represent standard deviation, n>600.

images showed spherical particles with smooth surfaces. The encapsulation efficiency of the RHO loaded particles were 2.61±1.12% and 6.96±1.32% for the 1000rcf and 3000rcf, respectively. The PTX loaded particles had higher encapsulation efficiencies of 45.1±9.95%and for 55.23±8.88%1000rcf and 3000rcf, respectively. A 7-day release study showed a cumulative release of 0.96% and 0.29% for 1000rcf and 3000rcf RHO-PCL. Burst release was observed in the first 24hrs with 0.79% and 0.23% released for 1000rcf and 3000rcf, respectively. Blank particles collected at both 1000rcf and 3000rcf did not affect cell viability after 24hrs or 48hrs of exposure, determined from the MTT assay. The free PTX, 1000rcf, and 3000rcf PTX loaded particles had little effect on cell viability in the PTX-resistant Ishikawa H cell line over 24hrs and 48hrs. In contrast, KLE cells are sensitive to PTX and had a greater response to both free and encapsulated PTX than the Ishikawa H cells. Quantitative cellular uptake revealed that the Ishikawa H cells had quicker uptake of the particles than the KLE, which is consistent with their sufficiency as transfection hosts.

Conclusions: Sequential centrifugation successfully separated PCL particles based on their size and removed any unreacted polymer. While there was a very slow release of RHO from the particles, it shows that the PCL particles would make a good drug delivery system for sustained release. While the PTX particles had limited effect on the Ishikawa H cell viability and a greater effect on KLE cell viability, the blank particles showed no effect at all in either cell line, again demonstrating that PCL is a good drug delivery system.

References: 1) Schatz-Siemers, N., Appl Immunohistochem Mol Morphol, 2020, 28(6) 453-459. 2) Siegel, R. L., et al., CA Cancer J Clin, 70(1), 7-30, 2020. 3) Weaver, B.A., Mol Biol Cell, 25(18), 2677-2681, 2014.