Isolation of Poly(caprolactone) Nanoparticles in an Effective Size Range for Endometrial Cancer Drug Delivery

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Statement of Purpose: Endometrial cancer (EC) is the most common female reproductive cancer worldwide, with a surgical hysterectomy being the predominant treatment option [1]. Polymeric nanoparticles are a promising drug delivery vehicle for therapeutic agents, with potential for more localized drug release. Poly(caprolactone) (PCL) is FDA approved and has been widely studied for its use in implantable biomaterials and drug delivery systems [2]. PCL is both biocompatible and biodegradable, and forms nano- and microparticles [2]. PCL particles can be loaded with Rhodamine B (RHO), a fluorescent dye, to aid in understanding of particle drug loading and release. Single emulsion solvent evaporation is a promising method for synthesizing polymeric nanoparticles; however, particle isolation using centrifugation of large volumes of the emulsion hinder the capture of particles < 500 nm in diameter. For systemic drug delivery, favorable nanoparticle diameters are < 200 nm in diameter [3]. We hypothesize using sequential centrifugation coupled with small emulsion volumes to maximize capture of particles in an appropriate size range for systemic delivery to EC.

Methods: Unloaded and RHO-PCL particles were synthesized using a single emulsion solvent evaporation (o/w) method. The aqueous phase consisted of 2.5% (w/v) PVA (MilliporeSigma, Burlington, MA) in DI water and the solvent phase consisted of 60 mg of 80,000 MW PCL (MilliporeSigma, Burlington, MA) in DCM (VWR, Radnor, PA). When preparing RHO-PCL particles, the solvent phase was supplemented with 3 mg RHO (MilliporeSigma, Burlington, MA). Large volume (LV) sequential centrifugation with 40 mL of emulsion was used to isolate particles of various size ranges. Small volume (SV) centrifugation with 2 mL emulsion samples was used at higher centrifugation speeds to improve isolation and yield of smaller particles. Particle size characteristics were visualized using SEM imaging. Dynamic Light Scattering was used to determine colloidal stability and Laser Dopper velocimetry was used to determine the surface charge of particles in 10 mM NaCl (RPI, Prospect, IL). To study the release kinetics of RHO from the particles, 5 mg/mL of lyophilized particles in DI water were added to a dialysis tube, submerged in 5 mL of a 1% (v/v) Tween 80 (RPI, Mount Prospect, IL) in DI water release media, and placed on an incubated shaker at 37 ºC and 350 rpm. The dialysis tube was placed in 5 mL new release media at various timepoints over a 1-week period and the collected release media was used to quantify the concentration of RHO released using a fluorescence plate reader. Cellular uptake of RHO-PCL particles was tested in Ishikawa, KLE, AN3CA and HEC-1A EC cell lines. Cell lines were incubated with a range of nanoparticle concentrations for 4 and 24 hours and the intracellular RHO was measured through the cell lysates obtained from 0.1% Triton-X (RPI, Mount Prospect, IL) solution. The concentration of RHO internalized by cells was quantified using a fluorescence plate reader.

Results: SEM imaging confirmed sub-micron spheres formed for both unloaded (Fig. 1A) and RHO-PCL formulations. Sequential centrifugation decreased the average size of particles collected from the emulsion. LV centrifugation at 10,000 rcf collected unloaded particles with an average diameter of 788 nm. SV centrifugation at 10,000 rcf significantly decreased the size of unloaded particles collected to 213 nm diameter. Release studies of RHO-PCL particles revealed effective RHO loading during synthesis and release, with a burst release within the first 12 h, followed by sustained release thereafter (Fig. 1B). RHO-PCL particles had similar zeta potential in solution, regardless of size (Fig. 1C). The average hydrodynamic diameter increased with increasing centrifugation speed, likely due to aggregation of particles during collection (Fig. 1D). EC cell lines had greater cellular uptake of RHO-PCL particles after 4 h compared to 24 h incubation at all tested particle concentrations (Fig. 1E).

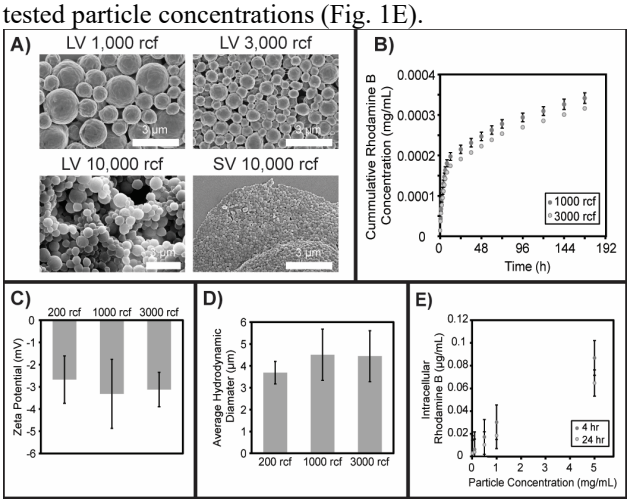

Figure 1. A) Unloaded PCL particles from emulation LV at 1,000 rcf, 3000 rcf, and 10,000 rcf and SV at 10,000 rcf. Characterization of RHO-PCL particle B) release in physiological solution, C) surface charge, D) colloidal stability, and E) Ishikawa cellular uptake after 4 and 24 h incubation.

Conclusions: Single emulsion solvent evaporation was successful in synthesizing spherical micro/nanoparticles in solution. LV centrifugation was effective in capturing microparticles, but SV centrifugation was more effective for collecting nanoparticles in a physiological size range for systemic delivery. RHO was successfully encapsulated in PCL particles and was effectively released, with burst and sustained release profiles, in aqueous solution over a 1-week period. Cellular uptake was successful *in vitro* within 4 h at various concentrations. These results are promising for developing a PCL particle formulation for drug delivery as a localized therapeutic for EC.

References: [1] Kuhn, TM, Curr. Oncol., 2023, 30(9), 7904-7919. [2] Espinoza, SM, Int. J. Polym. Mater., 2019, 69(2), 85-126. [3] Hoshyar, N, Nanomedicine, 2016, 11 (6), 673-692.