Characterizing pH-Responsive Nanoparticles for Treatment of Mesothelioma Malignancies

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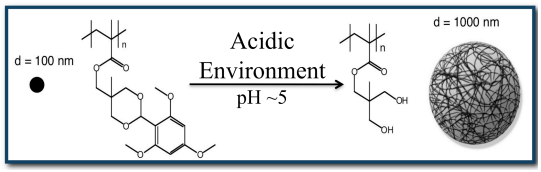
Statement of Purpose: Nanoparticles are widely studied as drug delivery devices because of their potential to increase drug solubility, alter biodistribution, target specific sites in the body, and minimize drug side effects[1]. We are investigating a unique pH-responsive nanoparticle for treatment of recurrent cancers[2], specifically mesothelioma malignancies; our strategy is to deliver a potent dose of drug to the surgical resection site thereby preventing tumor recurrence with high efficacy and low systemic exposure[3]. Fully characterizing the nanoparticles' mechanism of action, from cellular uptake to nanoparticle swelling, drug release, and cell death (Figure 1) is essential to future clinical use of these particles. To that end, we have conducted studies that support this expansion mechanism and provide further characterization of this polymeric delivery system.



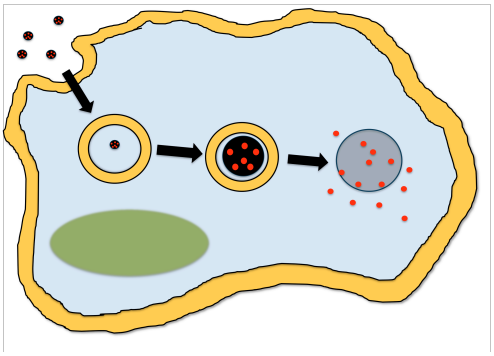


Figure 1. (bottom) Mechanism of delivery for pH-responsive expansile nanoparticles: 1) nanoparticles (black dots) are taken up into cell by endocytosis; 2) nanoparticles swell under the acidic conditions (pH ~5) of the endosome; 3) nanoparticles release their cargo intracellularly. **(top)** Action of nanoparticle swelling: the polymer protecting group is cleaved at a pH of ~5 to yield hydroxyl groups; this results in the transformation from a hydrophobic to hydrophilic polymeric structure. Nanoparticles consequently absorb water and swell (not to scale).

Methods: Nanoparticles were prepared using standard mini-emulsion polymerization techniques[4]. To study nanoparticle swelling and morphology, particles were exposed to acidic conditions (pH 5) for 24 h and their resultant diameter measured via dynamic light scattering (DLS), freeze-fracture TEM (ff-TEM), and qNano nanopore technology from IZON Ltd. Larger nanoparticles (~500 nm) were synthesized with a Rhodamine co-monomer for visualization using laser excitation and light microscopy. To confirm nanoparticle function (i.e. swelling) *in vitro*, mesothelioma tumor cells (MTSO-211H) were cultured with 100 nm diameter nanoparticles for 24 h and then examined using histological staining and TEM imaging. Effective delivery of the nanoparticle cargo, paclitaxel, was confirmed by culturing MTSO-211H cells with paclitaxel-loaded nanoparticles, empty nanoparticles, and free paclitaxel.

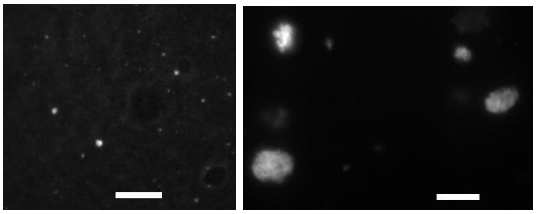


Figure 2. Light microscopy images of expansile nanoparticles after 24 h exposure to pH 7 **(left)** and pH 5 **(right)**. Scale bar = 5 μm.

Results: Characterization of the nanoparticle swelling mechanism showed that nanoparticles swell, on average, to between 3X and 10X their original size upon exposure to pH 5 for 24 hr (Figure 2, additional data not shown). Swollen nanoparticle morphology is typically spherical but a wide range of structures may exist. Cellular uptake of 100 nm diameter nanoparticles readily occurs within hours with subsequent particle swelling. Histological examination and TEM imaging of nanoparticles cultured with tumor cells revealed large polymeric bodies within cells. These bodies were ~1 μm in diameter, which is consistent with the expected swollen size of 100 nm particles (~10X the original size of the nanoparticles applied to the cells); these bodies did not appear in control samples grown without nanoparticles. Culturing of paclitaxel-loaded nanoparticles with MTSO-211H tumor cells revealed dose dependent decreases in cell viability consistent with the administration of free paclitaxel while empty nanoparticles had no effect.

Conclusion: pH-responsive expansile nanoparticles are a novel drug carrier device with a reliable trigger (pH) and mechanism of action (swelling). Both microscopic and macroscopic investigations confirm this mechanism of action. Additionally, introduction of nanoparticles into a cellular environment does not adversely affect the particle functionality. The decrease in cell viability subsequent to administration of paclitaxel-loaded nanoparticles to tumor cells also suggests that these particles effectively release therapeutic levels of drug.

References:

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