Paclitaxel-Eluting Expansile Nanoparticles for the Treatment of Breast Cancer

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Statement of Purpose: The use of nanoparticles in the diagnosis and treatment of cancer at the cellular level offers several significant advantages. Nanoparticles allow reduced dosage, ensure appropriate pharmaceutical effects, minimize side-effects, protect drugs against degradation and enhance drug stability[1-3]. Our interests lie in the use of pH-responsive polymeric nanoparticles to advance the current standards in breast cancer treatment. We have therefore designed nanoparticles that swell when exposed to an acidic environment and are thus termed expansile nanoparticles (eNPs). With this drug delivery system, we aim to provide local tumor control by targeting microscopic residual disease remaining after an initial surgical breast cancer intervention, and to eliminate early metastatic disease within regional lymph nodes. Herein, we report the *in vitro* efficacy of paclitaxel loaded expansile nanoparticles (Pax-eNPs) against human breast adenocarcinoma cells, the kinetics of cellular uptake of the eNPs, and the *in vivo* efficacy of the Pax-eNPs in a murine establishment model of human breast cancer.

Methods: Nanoparticles were prepared using a miniemulsion polymerization technique, which combines high energy emulsification and base-catalyzed free radical polymerization of an acrylate monomer and crosslinker[4]. After dialyzing the eNPs to remove excess surfactant and salts, we performed cell cytotoxicity experiments with paclitaxel-loaded and unloaded nanoparticles against a human breast adenocarcinoma cell line (MDA-MB-231). We also quantified cellular uptake of the particles via flow cytometry using 100 nm eNPs covalently modified with rhodamine. For our initial *in vivo* studies, we established a subcutaneous mammary fat pad tumor model, wherein we injected 2 x 10^6 luciferase-transfected MDA-MB-231 cells, followed by injection of treatment: 100 µg paclitaxel equivalent of Pax-eNP, unloaded eNP, 300 µg Pax IP or saline control. Disease progression was then monitored in all animals over a period of 4 weeks before final tumor evaluation.

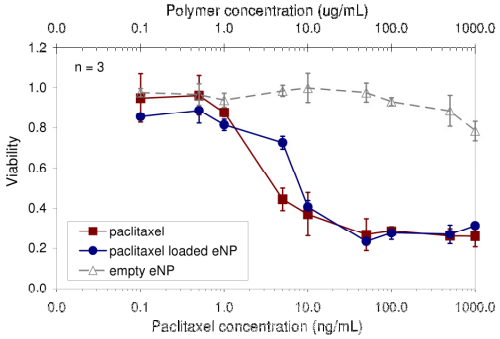

Figure 1. Percent relative viability of MDA-MB-231 cells following 72 hours of exposure to paclitaxel, empty eNP and paclitaxel-loaded eNP.

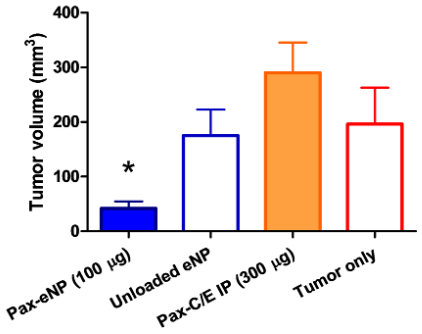

Figure 2. Tumor volume at 4 weeks for various treatment groups in a murine breast cancer mammary fat pad tumor model.

Results: Expansile nanoparticles have been synthesized from a pH-responsive monomer to afford smooth, spherical particles ~100 nm in diameter. Upon loading the eNPs with paclitaxel, we observed a dose dependent decrease in cell viability with an IC_{50} of ~10 ng/mL (Figure 1). Co-culture of MDA-MB-231 cells with unloaded eNPs did not result in tumor cytotoxicity, demonstrating that cell death was due to paclitaxel release and not exposure to the polymer itself. By using flow cytometry, we also monitored the internalization and accumulation of rhodamine-eNPs within the cells over time (data not shown). Finally, *in vivo* studies showed increased efficacy with the paclitaxel-loaded nanoparticles compared to paclitaxel alone and unloaded nanoparticles in preventing the establishment of breast cancer in mammary fat pads in mice (Figure 2).

Conclusions: In summary, we have synthesized smooth, spherical polymeric nanoparticles using a mild, base-catalyzed miniemulsion polymerization technique. The paclitaxel-loaded nanoparticles were readily taken up by MDA-MB-231 breast adenocarcinoma cells and are cytotoxic *in vitro* against this human cell line. Initial *in vivo* studies showed increased efficacy with the paclitaxel-loaded nanoparticles compared to paclitaxel alone in a murine establishment model of human breast cancer, suggesting that such technology may be useful in the prevention of microscopic residual disease recurrence after surgical resection.

References:

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