Effect of Transport Inhibitors on Nanoparticle Distribution in Human Breast Cancer Cells

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Introduction: Photothermal therapy (PTT) is a promising technique for cancer treatment that is more localized and less invasive than other methods such as radiation and chemotherapy. Laser-mediated photothermal ablation of cancer cells with nanoparticles can result in irreversible cancer cell damage and potential tumor regression. We hypothesize that pulsed laser PTT will yield higher efficacy when nanoparticles are accumulated on the cell membrane, as localized heating will disrupt membrane intactness. Previous studies have illustrated various mechanisms for cellular uptake of nanoparticles. Here, we used transport inhibitors to examine their effect on nanoparticle distribution within human breast cancer cells.

Materials and Methods: MDA-MB-231/green fluorescent protein (GFP) breast cancer cells were cultured in Dulbecco's Modified Eagle Medium with 5% fetal bovine serum, 1% Pen-Strep, 1% L-Glutamine and 1% Minimal Essential Medium amino acids at 37°C and 5% CO_2. We first performed cell viability tests via trypan blue 0.4%; cells were incubated for 30 minutes with a transport inhibitor: cytochalasin A, chlorpromazine, or nocodazole. Bright-field images of stained cells were taken at different inhibitor concentrations to find viable dosages. To image nanoparticle internalization, cells were seeded to a 12-well plate at 100,000 cells per well. After becoming confluent (~24 hours later), cells were incubated with each inhibitor for 30 minutes and then incubated with PLA-PEG nanoparticles loaded with ICG (indocyanine green) and Nile Red at 267 μg/ml for 30 minutes. Average nanoparticle size was around 100 nm and nanoparticles fluoresced with an emission peak at 625 nm when excited at 545 nm due to the Nile Red fluorophore. Cells were imaged with a two-photon microscope and images were processed with ImageJ to combine channels. Channel 1 displayed the nanoparticles and channel 2 displayed the cells with GFP; emission wavelengths were 560-630 nm and 500-550 nm respectively.

Results and Discussion: Cells treated with 10 μg/mL chlorpromazine showed partial nanoparticle accumulation on the cell membrane (Fig. 1), but higher concentrations did not yield improved results. For control samples, particle aggregation in the cytoplasm increased as the nanoparticle concentration increased. Microfilament disruptors such as cytochalasin A and nocodazole (TLC) did not prevent cellular nanoparticle internalization. Increasing inhibitor concentration resulted in a trend of decreased cell viability.

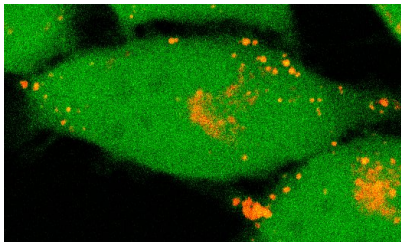

Figure 1. Cells treated with 10 μg/mL chlorpromazine show nanoparticle accumulation on the cell membrane.

Conclusions: The results suggest that at 10 μg/mL chlorpromazine can cause nanoparticle accumulation on the cell membrane. However, cytochalasin A and nocodazole (TLC) do not significantly affect nanoparticle uptake for these human breast cancer cells, as many nanoparticles were internalized and found in the cytoplasm. Further study is needed to determine if there is a significant difference in efficacy of pulsed-laser treatment when nanoparticles are located primarily along the cell membrane as opposed to being distributed within the cytoplasm.

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