IR820-Loaded Poly(lactic-co-glycolic acid) Nanoparticles for Photothermal Cancer Therapy

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Statement of Purpose: Photothermal therapy (PTT), which utilizes nanoparticles embedded in tumors as exogenous energy absorbers to convert externally applied near-infrared (NIR) light into heat to ablate cancer cells, has shown promise as an alternative strategy to treat cancer[1-3] but it typically uses gold-based nanoparticles that will remain in the body for extended periods of time with unknown long-term health effects. To maximize the safety of PTT, we have developed poly(lactic co-glycolic acid) (PLGA) nanoparticles (NPs) encapsulating the NIR-absorbing dye IR820 as a biodegradable platform for PTT. We evaluated the efficacy of these IR820-PLGA NPs in triple negative breast cancer (TNBC) cells. TNBC accounts for 15-25% of all breast cancers and it lacks expression of the three most common receptors seen on other breast cancer subtypes.[3] This lack of expression makes TNBC unsusceptible to current targeted or hormonal therapies, so new treatment strategies such as PTT are needed to combat this disease. The results presented here show that IR820-loaded PLGA NPs are excellent mediators of PTT for potent treatment of TNBC.

Methods: *IR820-PLGA NP synthesis & characterization:* IR820-loaded PLGA NPs were prepared by the oil-in-water single emulsion solvent evaporation method.[4] A solution of PLGA and IR820 was added dropwise to water, stirred to allow solvent evaporation, then purified by centrifugal filtration. The NPs' size, surface charge, IR820 encapsulation efficiency, and stability in storage conditions (4°C in water) and more physiologically relevant conditions (37°C) were evaluated using dynamic light scattering (DLS), zeta potential, transmission electron microscopy (TEM), and spectrophotometry measurements. The temperature increase of free IR820 and IR820-PLGA NPs suspended in PBS during laser irradiation (808 nm, 5 min, 1.5 W/cm^2) was measured using an FLIR thermal camera. *In vitro studies:* Uptake of free IR820 or IR820-PLGA NPs by MDA-MB-231 TNBC cells was assessed by flow cytometry, and each treatment's cytocompatibility was measured with an MTT assay. The potency of PTT mediated by free IR820 or IR820-PLGA NPs was determined by exposing MDA-MB-231 cells to either treatment with or without laser application (808 nm, 5 min, 1.5 W/cm^2), then performing an MTT assay or flow cytometry after staining the cells with calcein AM and ethidium homodimer-1 (EthD-1).

Results: *IR820-PLGA NP characterization:* DLS, TEM, and zeta potential measurements revealed the IR820-PLGA NPs were spherical and monodisperse with a hydrodynamic diameter of 59.6±12.6 nm and a surface charge of -39.9±6.1 mV. Spectrophotometry showed that IR820 was encapsulated at >90% efficiency and retained inside the PLGA NPs after an initial burst release of 30% within the first 30 minutes of suspension in water, which we attribute to loss of adsorbed dye from the exterior of the NPs. The NPs were stable (no change in size, surface charge, or IR820 loading) when suspended in water and stored at 4°C for one month. When kept at 37°C, IR820 dye began to release after 20 days and particle size increased. Importantly, thermal imaging demonstrated that IR820 encapsulated in PLGA NPs heats as well as free IR820. *In vitro studies:* Flow cytometry showed that free IR820 and IR820-PLGA NPs enter MDA-MB-231 cells in a dose- and time-dependent manner. An MTT assay revealed that free IR820 at concentrations >10 μM is toxic to cells, but encapsulation in PLGA increases the cytocompatibility of the dye such that doses of 35 μM can be safely applied. Irradiation of cells treated with these respective concentrations of free IR820 and IR820-PLGA NPs caused a decrease in metabolic activity (Fig. 1), and IR820-PLGA NPs provided more potent PTT.

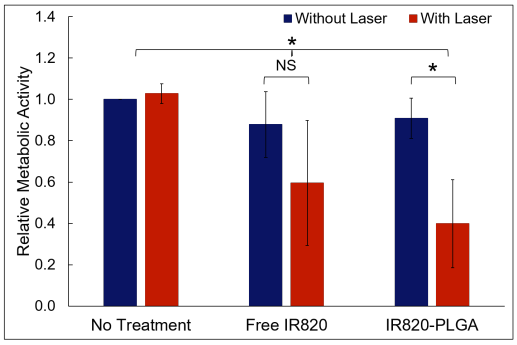

Figure 1. PTT mediated by IR820-PLGA NPs reduces MDA-MB-231 metabolic activity to a greater extent than PTT mediated by free IR820. *p < 0.05

Flow cytometry also showed an increase in EthD-1+ cells (dead cells) and a reduction in Calcein AM+ cells (live cells) when samples were treated with PTT mediated by IR820-PLGA (Fig. 2). The percentage dead cells was greater than that produced with free IR820, indicating IR820-PLGA NPs are an effective platform for PTT.

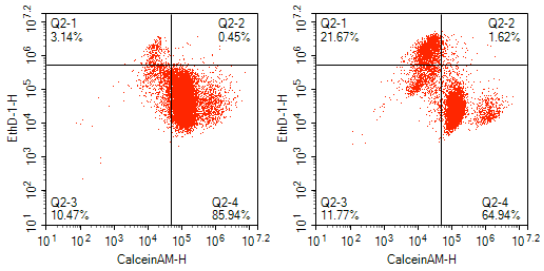

Figure 2. A higher percentage of cells treated with IR820-PLGA and irradiated with NIR light (right) are positive for EthD-1 than untreated control cells (left).

Conclusions: IR820-PLGA NPs can enable potent PTT. These data support continued development of this platform for treatment of aggressive cancers like TNBC that are unsusceptible to conventional treatments.

References: 1. Fay BL, *et al.* Int J Nanomedicine. 2015;10:6931-6941. 2. Riley RS, Day ES. WIRES Nanomed Nanobiotechnol. 2017;e1449. 3. Bianchini G. Nature Rev Clin Onc. 2016;13:674-690. 4. Srinivasan S. J Photochem Photobio B. 2014;136:81-90.