Evaluating Cellular Lethality for Treatment of Melanoma by Photothermal Therapy

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Introduction: The American Cancer Society predicted that approximately 96,480 people will be diagnosed with melanoma skin cancer this year, and 7,230 of them will die [1]. Minimally-invasive alternatives for melanoma treatment are a clinical need, and a continued unmet need exists for combinatorial therapies with limited toxicity and/or resistance profiles. Photothermal therapy (PTT) can be used as a non-invasive treatment by delivering targeted nanoparticles and a laser source (typically in the near-infrared range) to the tumor site. We propose to use a biodegradable nanoparticle platform based in polymers to reduce the toxic risks. Our goal is to evaluate the cellular lethality of nanoparticles on melanoma cells as a response to dosimetry using an *in vitro* model.

Materials and Methods: Poly(lactic acid)-methoxy-poly(ethylene glycol) (PLA-mPEG) nanoparticles were made through a precipitation process with materials that were previously used in FDA-approved drug delivery systems. The nanoparticles were loaded with indocyanine green (ICG) as a photothermal agent to induce hyperthermia. The particle size distribution was measured with dynamic light scattering and the optical absorbance with absorption spectroscopy. We performed PTT on B16-F10 murine metastatic melanoma cells. The cells were placed in a 96-well plate at 20,000 cells/well and incubated for 24 hours at 37°C with 5% CO_2. Then, we added 1.6 mg/mL concentration of PLA-mPEG nanoparticles. The cells were exposed to different fluence rates from 0.79 to 4.77 W/cm^2 for 3 minutes using a near-infrared diode laser of 808 nm to induce hyperthermic temperatures. We used an infrared camera to measure the temperature of the cells. An hour after irradiation, we analyzed cell viability using the MTS assay (cells were incubated with 10 µl of MTS reagent for 2 hours). The experiments were performed with four control groups: negative control (no laser; no nanoparticles), dark control (no laser; nanoparticles dose), sham control (irradiation laser for 3 min; no nanoparticles), and positive control (methanol for 5 min). Analysis of variance (ANOVA) was used for statistical comparison of the means of the various samples.

Results and Discussion: We prepared sub-100 nm nanoparticles with mean sizes of ~75 nm. The optical absorbance showed a peak for ICG at 788 nm. Using the laser, the temperature change for the sham control was <1.5°C, and for the treatment groups, it increased up to ~9°C for the low fluence rate and up to 20°C for the high fluence rate. From the MTS assay, the cell viability with respect to negative control for dark control was ~100%, for sham control with low fluence was 100% and with high fluence was ~80%, and for positive control was ~6%. For the treatment groups, the cell viability decreased as the laser fluence increased. It was ~86% for low fluence and ~41% for high fluence. Figure 1 shows the cell viability as a function of the maximum temperature reached and thermal dose (temperature and irradiation time) in terms of cumulative number of equivalent minutes at 43°C (CEM43°C) [2].

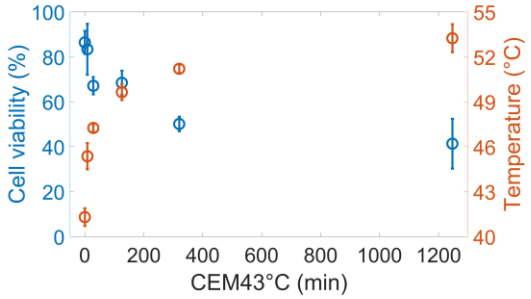

Figure 1. Cell viability in terms thermal dose (CEM43°C) and maximum temperature reached.

Conclusions: Our results demonstrate that using the PLA-mPEG nanoparticles generates hyperthermia and cell death. PTT has been shown to alter immune responses, so future directions include investigating the impact of the thermal dose with induced immunogenic cell death and the release of damage associated molecular patterns.

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References:

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