Ruthenium-complex Nanomicelle Sensors for Reporting Oxygen Tension within the 3D Tumor Model Microenvironment

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Statement of Purpose: Oxygen tension and hypoxia play an essential role in oncogenesis and tumor progression. It has been shown that tumor hypoxia correlates with poor prognosis of cancer patients, including breast, lung, and head and neck cancers, as it may enhance the resistance to chemotherapy and radiotherapy. Previous methods to measure oxygen tension (pO_2) within patient tumors and 3D tumor models have utilized micro-oxygen electrodes. These have been the gold standard to measure pO_2; however, they lack high spatial resolution and consume oxygen in the process. In contrast, an imaging technique termed two-photon phosphorescence lifetime microscopy (2P-PLIM) in conjunction with oxygen sensing optical probes can permit oxygen measurements with high spatial resolution and high sensitivity in biologically-representative tissue culture models with no oxygen consumption in the process.

The goal of our study is to encapsulate the hydrophobic, two-photon excitable oxygen sensor $[Ru(dpp)_3]^{2+}$ (Ru) within a polymer micelle to create hydrophilic Ru-complex micelles and incorporate these micelle sensors within 3D matrices containing multicellular spheroids. We aim to characterize these micelle complexes and implement them to report pO_2 within our spheroid models.

Methods: Preparation of Ru-complex micelles was performed similarly as reported by Khan et al.[1] Briefly, 4 mg of $[Ru(dpp)_3]Cl_2$ was dissolved in 0.5 mL of methanol and dried to form a thin film. 100 mg of poloxamer-407 was added to the film, followed by addition of 10 mL deionized water. Micelles were formed via ultrasonic homogenizer with a 3.4 mm tip that was continuously run for 10 second intervals of cycling on and off for a total of 120 seconds at 50% amplitude. The mixture was filtered via Amicon Ultra-15 filter with a 10 kDa cutoff and centrifuged at 5000 g for 30 minutes. The subnatant was collected and analyzed on a plate reader (Tecan) with known standards to determine encapsulated fraction of Ru within the micelles via fluorescence readouts. Micelle size was characterized via nanoparticle tracking analysis (NTA) as well as dynamic light scattering (DLS). Localization of micelles within and surrounding breast epithelial (MCF10A) spheroids was performed via two-photon microscopy to image collagen by second harmonic generation (SHG) as well as Ru fluorescence imaging. Viability was assessed via Hoechst and ethidium homodimer staining of MCF10A spheroids at various concentrations of Ru-complex micelles in the media.

Results: The Ru-complex micelles created were soluble in water with a mean encapsulation efficiency of 93 ± 1.5 %. NTA revealed that the mean diameter of the Ru-complex micelles was 124 nm (Figure 1A). DLS determined a similar mean diameter of 133 nm. To visualize the localization of the Ru-complex micelles, fluorescent imaging of Ru was performed in 1 mm thick collagen gels embedded with MCF10A spheroids. Ru-complex micelles were either added to the gel mixture prior to collagen gel formation or in the media after the gels polymerized. When the Ru-complexes micelles were added in the gel mixture, SHG imaging after 16 hours showed that collagen structure was disrupted but Ru fluorescence was present in the perimeter of the spheroids. In contrast, Ru-complex micelles added to media showed no disruption in collagen structure as well as localized fluorescence signal within and surrounding spheroids (Figure 1B). Signal remained after 1 week of culture. MCF10A spheroid viability analyses were conducted with Ru-complex micelles added to media at various concentrations. MCF10A spheroids with Ru-complex micelle concentrations of ~7.5 µM were similar to a control absent of micelles with mean viability of about 85 %. Viability decreased to around 47 % with Ru-complex micelle concentration of ~150 µM.

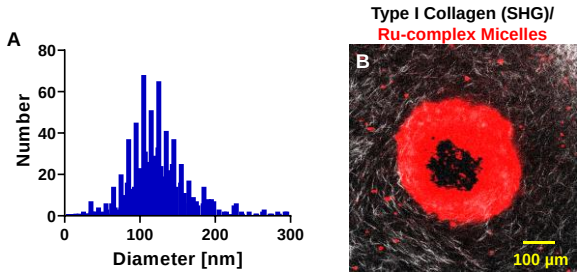

Figure 1. Characterization and imaging of Ru-complex micelles. **A.** Histogram of micelle diameters measured by nanoparticle tracking analysis. **B.** Two-photon microscopy image slice of embedded breast epithelial (MCF10A) spheroids. Image shows type I collagen via SHG as well as fluorescence from Ru (red) within and surrounding the spheroid.

Conclusions: Hydrophobic Ru was successfully encapsulated to create hydrophilic complex nanomicelles that can be visualized within MCF10A spheroids while maintaining high viability. Future work will aim to report oxygen tension from the Ru-complex micelles in hydrogel-based 3D spheroid cultures utilizing 2P-PLIM. This method to spatially resolve oxygen concentrations in real time will allow for future development and expansion of the platform to determine pO_2 gradients and sites of hypoxia within our spheroid models, including breast cancer spheroids.

References:

[1] Khan A. et al., RSC Advances, 2015;291-300.