Design of a Tissue Engineering System to Influence and Quantify Oxygen Gradients

Erik Bland, Karen J.L. Burg
Institute for Biological Interfaces of Engineering, Clemson University

Statement of Purpose: Tissue engineering successes have been largely limited by, among other factors, the ability to develop vasculature and thus provide nutrients for cells seeded within an engineered scaffold. Injectable composites[1] offer a less invasive implantation option, conform to irregular tissue defects, allow good mass transport for nutrients, and may be closely matched to the mechanical properties and hydration state of soft tissues. To begin the construction of a viable "large" volume injectable composite, one must design for adequate oxygen diffusion and convection. The objective of this work is to develop a model that allows quantitative measurement of spatial oxygen partial pressure, and thus provides an analysis of cellular response to hypoxia in a tissue engineering environment. Measurement of spatial oxygen partial pressure requires a sensing mechanism that can be used for 3D measurement, gives real-time response, does not affect the quantity measured, and is non-toxic to cells. For this purpose, novel O_2 sensitive nanoparticles[2,3] have been tested. These sensors work by an oxygen-dependent fluorescence quenching mechanism such that fluorescence intensity increases as local pO_2 decreases.

Methods: Oxygen sensitive nanoparticles using a variety of sensing moieties (poly-dihexylfluorene (PDHF), ruthenium, and porphyrin based compounds), and of varying particle sizes (roughly 20nm and 200nm), were obtained. Nanoparticles were provided without buffer in aqueous solution, at a concentration of approximately 5 ppm for Ru and PDHF and 10ppm for porphyrin-based sensors. Nanoparticles were tested for cytotoxicity against murine fibroblasts (3T3) at varying concentrations over a span of 3 days. Briefly, cells were seeded onto 24-well culture plates in the amount of 25,000 cells per well and allowed to attach overnight. Next, the culture medium was aspirated and replaced with sensor solution mixed with concentrated DMEM to a final DMEM concentration of 1X. Cells lysed at each time point using Triton X-100 served as positive controls. Cells grown in media without sensors served as negative controls. Viability was assessed, following addition of the sensors at Days 1, 2, and 3, by light microscope and by Live/Dead and LDH (lactate dehydrogenase) cytotoxicity assays.

Results: The first cytotoxicity study compared Ru (silica coated, approx. 200nm size), PDHF (silica coated), and PDHF (uncoated, approx 20nm size) sensors. The purpose was to determine if the silica coating prevented toxicity caused by Ru leaching or if the silica coating was cytotoxic. Assessment by microscope yielded two different cases: (1) attached fibroblasts in abundance with normal, healthy, 'spread' morphology, and (2) large numbers of detached, floating, round cells. These results correlated well with the LDH cytotoxicity data shown in Figure 1. A higher absorbance correlates to a higher number of dead or damaged cells and sensor concentration (ppm) is given on the x-axis. Red indicates

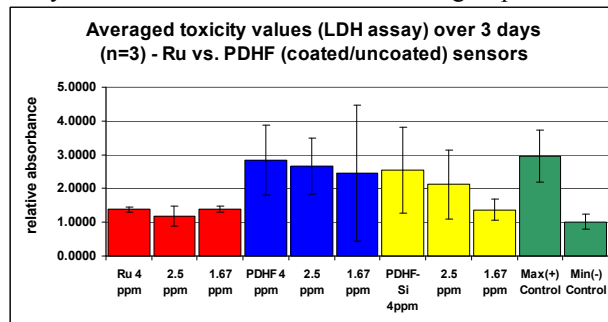the Ru group, blue indicates the PDHF (uncoated) group, and yellow indicates the Si-coated PDHF group.

Figure 1 – Ru and PDHF coated and uncoated sensors

Next, a more sensitive, stronger fluorescing sensor was provided, based on a porphyrin oxygen sensitive moiety. The toxicity test was run again, comparing large (~200nm) and small (~20nm) porphyrin-based sensors. The LDH data is shown below in Figure 2, below. S (red) and L (blue) indicate small and large sensors respectively.

Figure 2 – Large and small porphyrin sensors
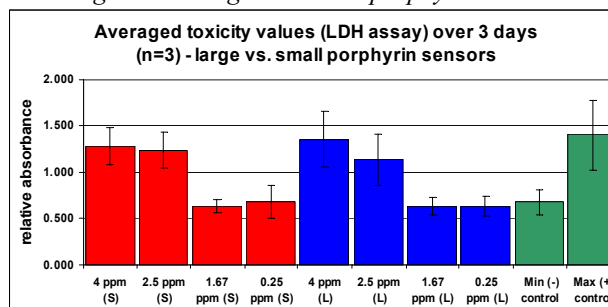
Conclusions: The cytotoxicity results suggest that, for the specifications tested, sensor toxicity is not correlated to the silica coating or Ru leaching and is not size dependant. The porphyrin based oxygen sensors were found to be a valuable tool for 3D O_2 imaging. The porphyrin sensors were found to be non-toxic below concentrations of roughly 33% of provided stock concentration (10 ppm), and also exhibited high fluorescence intensity. Further work will include characterization of porphyrin sensors as a function of size with respect to long-term toxicity, response time to changes in pO_2, and feasibility of immobilization and imaging within a tissue engineering construct.

References:

1. Burg KJL et al. *IEEE Eng Med Biol*, 22(5), 2003.
2. Wu C et al. *Langmuir*, 22 (7), 2006.
3. Wu, C et al. *J. Phys. Chem. C,* 112(6), 2008.