Spatiotemporal Dissolved Oxygen Concentrations from a Nanofiber Cell Seeded Scaffold

Bowers DT[1], Tanes ML[1], Keane NA[1], Lin Y[1], Das A[1], Fraser CL[1] and Botchwey EA[1,2]
[1]University of Virginia, Charlottesville, VA, [2]Georgia Institute of Technology, Atlanta, GA

Statement of Purpose: The size of tissue engineering constructs is limited by the diffusion of oxygen and other nutrients from the nearest capillaries. This usually requires flat, thin constructs to keep cells within a couple hundred microns of the nearest capillaries. Many strategies exist to vascularize constructs, including local delivery of proangiogenic factors. Hypoxia is also an issue in cell culture conditions. The success of hypoxia battling techniques is often measured as a function of vascularity or survival of cells in culture. While these methods are essential to the assessment of strategies to combat hypoxia induced cell death, oxygen tension is a key underlying parameter. Oxygen sensitive boron dyes incorporated in polymer nanofibers can provide this information right where extracellular hypoxia is detrimentally affecting cell survival. Not only is this information provided from the scaffold where cells are attached, it is also provided without modification of the seeded cells.

This is demonstrated below with a novel formulation of the dye where the boron containing fluorescent dye is conjugated to a polylactic acid (PLA) polymer. The dye containing fibers are shown to be biocompatible. Furthermore, when layered on a base of poly(hydroxybutyrate-co-valerate) and polycaprolactone (PHBV&PCL) electrospun fibers, used for scaffold mechanical durability, the dye is shown to be reliably oxygen sensitive in both dry and aqueous environments. Finally, it is shown to be sensitive in the range of dissolved oxygen which allows the detection of hypoxia surrounding a monolayer of cells in culture.

Methods: BF_2dbm(I)PLA nanofibers were electrospun in a base solvent of methylene chloride. Calibration measurements were made using a custom set up allowing a chamber to be placed over the objective of an inverted microscope and images were quantified at two wavelengths. NIH3T3 cells were used for biocompatibility studies while D1 cells were used for oxygen measurements. Cell viability was assessed using propidium iodide and fluorescein diacetate.

Results: BF_2dbm(I)PLA nanofibers were electrospun using parameters optimized for low molecular weight PLA. Electrospinning solvent modifications including addition of ethanol and pyridinium formate were employed to obtain desired fiber morphology. These fibers were confirmed to be biocompatible by cell viability staining.

The boron dye is conjugated to a relatively (for electrospinning) low molecular weight polymer (15kDa) to give desired optical properties. However, the electrospun scaffold does not possess good mechanical durability. Therefore the BF_2dbm(I)PLA nanofibers were electrospun onto a layer of PHBV&PCL blended fibers, which are very flexible and durable. The ultimate tensile strength of these composite scaffolds is increased by about 0.24 MPa and the Young's modulus is increased by about 2.5 MPa by the addition of the boron dye to PHBV&PCL scaffold. Direct measurements of the dye nanofibers themselves were not possible because of the brittle nature of the mesh.

The phosphorescence of the dye is quenched by oxygen, while the fluorescence remains unchanged as a result of oxygen. Hence, the ratio of fluorescence to phosphorescence is calculated to detect the concentration of oxygen. In gaseous conditions, the fibers are shown to be responsive to oxygen concentration changes between 2 and 12%, where the fluorescence to phosphorescence ratio is approximately linear. These numbers do not change when the measurements are made through the PHBV&PCL base layer. No lag time between changing the oxygen concentration and a response from the scaffold could be detected.

In aqueous solutions the phosphorescence to fluorescence ratio was shown to follow a power function within the range of 0 to 20 ppm dissolved oxygen. This was the same in Phosphate Buffered Saline and Dulbecco's Modified Eagle Medium. The scaffold is able to sense cells cultured in a monolayer with 3 and 18% decreases in the phosphorescence to fluorescence ratio as the radial distance from the edge of the cell monolayer increases.

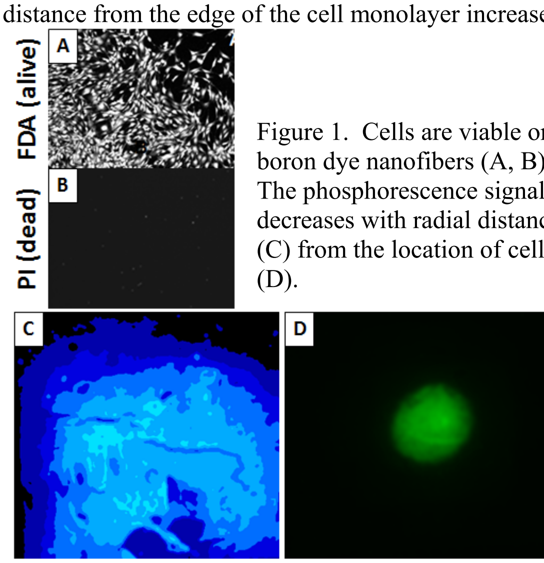

Figure 1. Cells are viable on the boron dye nanofibers (A, B). The phosphorescence signal decreases with radial distance (C) from the location of cells (D).

Conclusions: The polymer conjugated boron dye (BF_2dbm(I)PLA) can be electrospun into nanofibers which are functional when layered with a base of PHBV&PCL fibers. The phosphorescence to fluorescence ratio can be calibrated to the oxygen concentration in dry and aqueous environments. The internal standard of the dye fluorescence reduces the number of compounds required in the scaffold. These fibers are sensitive to oxygen concentrations which occur in monolayer cell culture. Therefore, these BF_2dbm(I)PLA nanofiber scaffolds are capable of providing real time spatial oxygen concentrations which can be used as a platform to study the effectiveness of hypoxia reducing strategies.