Oxygen-sensing Boron Biomaterials as a Platform for Tissue Engineering

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Statement of Purpose: While tissue engineering advances have brought us closer to the day when tissue engineered substitutes may replace many autologous transplantations across many fields of medicine, still lacking is an understanding of the limits of oxygenation in tissue engineered scaffolds and implants. Having a platform by which we could evaluate new therapies before moving to clinical trials could save significant time and costs to the laboratory researcher. To that end, we have developed a class of light emitting boron biomaterials which exhibit both fluorescence (F) and unusual room temperature phosphorescence (RTP). We expect these materials to be critical for creating oxygen-sensing scaffolds for tissue engineering applications, and also to serve as a platform for the examination of potential scaffolding materials and construction. Within these unique materials, F is a molecular probe also serving as an oxygen-invariant internal standard, allowing the RTP to function as a sensitive optical sensor for oxygen. To make this material functional, we have successfully fabricated nanofiber scaffolds through electrospinning blended difluoroboron dibenzoylmethane and poly(lactide co-glycolide) (PLGA). Electrospinning as a technique permits us to manipulate the process parameters and thereby affect fiber diameter and morphology. Initial studies demonstrated our ability to fabricate blended dye-polymer nanofiber meshes with 200nm average diameter, and from our knowledge of the process, we have the potential to fabricate fibers under 100nm in diameter. These dimensions mimic those of the extracellular matrix, and typically enhance cell attachment and growth over non-textured surfaces. When these blended dye-polymer constructs are exposed to UV light, they retain the characteristic F and RTP of the dye alone, suggesting our processing methods do not damage the dual emissive properties of the dye. With these materials we can collect ratiometric oxygenation information throughout a tissue space.

Methods: 3,6-Dimethyl-1,4-dioxane-2,5-dione (D,L-lactide, Aldrich) was recrystallized twice from ethyl acetate and stored under nitrogen. Boron polymers were prepared as previously reported (Zhang G. J Am Chem Soc. 2007;129:8942-3) and spectra were recorded on a Varian UnityInova spectrometer in CDCl3 unless otherwise indicated. UV-vis spectra were recorded on a Hewlett-Packard 8452A diode-array spectrophotometer in CH_2Cl_2. Molecular weights were determined by gel permeation chromatography (GPC) (THF, 25 °C, 1.0 mL/min) using multiangle laser light scattering (MALLS) (λ= 633 nm, 25 °C) and refractive index (RI) (λ = 633 nm, 40 °C) detection. To electrospin nanofibers, a dye-polymer blend solution was loaded into a syringe pump and dispensed with a designated flow rate through an 18 gauge needle. A voltage drop (10-20kV) was created between the needle tip and the collector using a DC power supply. Fibers were collected on a grounded aluminum plate for electron microscopy analysis or glass coverslips to determine luminescence.

Results: We successfully fabricated dye-polymer blended nanofibers with an average diameter of about 200 nm (Figure 1a). These blended nanofibers do not exhibit any of the beaded defects which often occur within electrospun fiber meshes (for example in the dye-free PLGA nanofibers shown in Figure 1b), making these ideal candidates as a drug delivery candidate with a consistent release, or as a consistent platform for determining oxygenation across a tissue space. Under exposure to UV light, dye-polymer blended nanofiber meshes maintain the dual emissive properties (Figure 1c), clearly illustrating that the dye maintains its phosphorescent and fluorescent properties after processing.

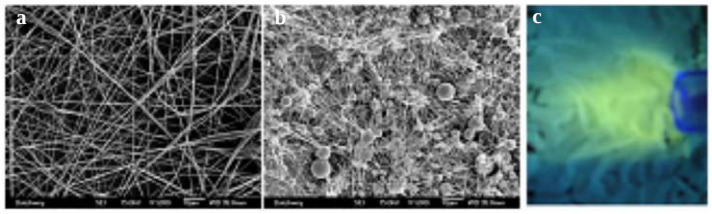

Figure 1. Scanning electron micrographs of electrospun PLGA nanofibers blended (a) and not blended with BF2dbmOMe dye (b). Qualitative observation of electrospun PLGA (85:15, 16% w/v) emission with halide substituted BF2dbm (5% w/w) on grounded aluminum collector while exposed to a hydrocarbon gas to displace oxygen (λ_{ex}= 365 nm) (c). Blue color on periphery indicates fluorescence. Yellow green color in oxygen-free gas stream corresponds to phosphorescence.

Conclusions: Nanofiber scaffolds are currently under investigation as drug delivering scaffolds, skin bandages and patches, peripheral nerve conduits, islet delivery scaffolds and other tissue engineering applications. A critical need for oxygen sensing in these applications could be met by our oxygen-sensing nanofiber scaffold, yielding immediate applicability in many areas of tissue engineering and regenerative medicine.

References: Zhang G, Chen J, Payne SJ, Kooi SE, Demas JN, Fraser CL. Multi-emissive difluoroboron dibenzoylmethane polylactide exhibiting intense fluorescence and oxygen-sensitive room-temperature phosphorescence. J Am Chem Soc. 2007 Jul 25;129(29):8942-3.