

Microfluidic bioreactors as tools for monitoring cell microenvironment

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Statement of purpose: Understanding the mechanisms underlying cell response to cues from their biomaterial microenvironment will ultimately lead to improved methods to control cell behavior in tissue replacement therapies. Whereas many methods have been established for characterizing cellular behavior and physical properties of biomaterial scaffolds, few methods exist for quantitatively mapping the fluctuations of soluble cues that impact cellular function. *Our overall goal* is to develop an integrated microfluidic bioreactor/sensor system to investigate cell response to changes in the chemical microenvironment found within 3D biomaterial scaffolds. *Our current work* focuses on developing novel technologies for sensing spatial and temporal changes in oxygen content during 3D culture. Oxygen is required for the aerobic metabolism of carbon compounds in cells and as such is a critical parameter (along with pH, temperature, and nutrient supply) that impacts cell viability. Furthermore, oxygen tension itself is a cue that directly impacts cell response (e.g., migration). Measurements of oxygen concentration in microfluidics systems are difficult because traditional oxygen-sensing approaches are not amenable to miniaturization. Moreover, oxygen-sensing electrodes also consume oxygen during operation. We present the development of an oxygen sensor based on fluorescence quenching of PDMS-embedded tris (4,7-diphenyl-1,10-phenanthroline) ruthenium (II) dichloride. By monitoring the quenching of this oxygen-sensitive fluorescent dye, measurements of oxygen concentration can be carried out in a non-invasive manner without consuming the oxygen available to the cells during culture. Results from microfluidic bioreactor fabrication and oxygen sensor calibration are presented.

Methods: *Fabrication of microfluidic bioreactor.* Soft lithography was used to fabricate the bioreactor as previously described by Whitesides and coworkers [1]. To fabricate the master, photoresist was patterned on 2.54 cm x 2.54 cm microscope slides. PDMS (20:1 elastomer:curing agent) was poured over the master and cured overnight. The PDMS replica was oxidized and sealed to a clean glass slide, also 2.54 cm x 2.54 cm. 0.58 mm-ID polyethylene tubing was connected via incisions made with a 1 mm blunt syringe (Fig. 1). *Oxygen sensor sample preparation.* PDMS was similarly prepared and cut into 3 cm x 1 cm x 0.2 cm pieces. The PDMS pieces were pre-swelled by submerging in dichloromethane for 10 min. Afterwards, the PDMS samples were submerged in a solution of 0.5 mM fluorescent ruthenium dye in dichloromethane for 3 or 9 min ($n = 3$ each). *Fluorescence measurements.* Fluorescence kinetics measurements were carried out in a Varian Cary Eclipse fluorescence spectrophotometer (470 nm excitation and 610 nm emission). Kinetics measurements were taken every 0.6 s for 1 h while the oxygen content of the atmosphere surrounding the samples was varied using mixtures of air and nitrogen gas (Fig. 2).

Results/Discussion: *Characterization of bioreactor.* A wide range of flow rates (from 0.001 to 5 mL/min) was achieved in the bioreactor using water. For flow rates up to 1 mL/min, Reynolds numbers ranged from 4.2 to 83.2, indicating laminar flow. Shear stresses at the walls of the microchannel ranged from 9.4 to 187.5 dyn/cm². *Sensor calibration.* The decrease in intensity of the fluorescent dye can be related to the concentration of the quencher by using the Stern-Volmer equation [2, 3]. The sensitivity [$S = (I_0/I) - 1$] was plotted with respect to oxygen content (assumed to be 21% in air) (Fig 3). Results shown in Fig. 3 are means +/- standard deviation. Data were compared using a Student's t-test. The values of sensitivity were considered significantly different if $p < 0.05$. The mean values of sensitivity for the 3-min and the 9-min samples were found to be not significantly different ($p > 0.05$).

Conclusions: A microfluidic bioreactor was fabricated using PDMS and glass slides. A wide range of volumetric flow rates was achieved under laminar conditions. We have also demonstrated the oxygen sensor system based on fluorescent ruthenium dye embedded in PDMS is able to detect changes in oxygen content of 0 to 21% in air. Current work focuses on monitoring oxygen content in liquid solutions and fabricating the microfluidic bioreactor with the oxygen-sensing ruthenium dye embedded in the channel walls. Future work focuses on determining cellular viability under normoxic and hypoxic conditions within the microfluidic bioreactor as well as 3D scaffolding biomaterials.

References: [1] Whitesides G.M., et al., *Electrophoresis*, 2000. **21**(1): p. 27-40. [2] Bacon, J.R and Demas, J.N., *Anal Chem*, 1987. **59**: p. 2780-2785. [3] Kneas K.A., et al., *Applied Spectroscopy*, 1997. **51**(9): p. 1346-1351.

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Figures: (1) Photograph of microfluidic bioreactor. Microchannel dimensions are 200 μm x 200 μm x 1 cm (width, depth, and length). (2) Schematic of kinetics experiment. (3) Sensitivity with varying oxygen content for 3-min and 9-min samples.

