Quantitative Fluorescence Imaging for Tissue Engineered Systems

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Statement of Purpose: Tissue engineering successes have been largely limited by, among other factors, the ability to develop vasculature and thus provide nutrients and oxygen for cells seeded within a scaffold[1-2]. Many measurement tools used in tissue engineering are based on 2D cell cultures or animal models, and are not completely amenable to spatial measurements in 3D tissue engineered scaffolds *in vitro*. The objective of this work was to develop optical methods for the quantitative, spatial measurement of oxygen and nutrient gradients and cell activity in 3D tissue engineered systems.

Methods: Methods to quantify fluorescence intensity were developed, using widefield fluorescence microscopy. Nanoparticulate oxygen sensors[3] were dissolved into agarose hydrogel matrices with cells and spatial imaging was performed to measure the oxygen gradients produced by cellular oxygen consumption. First, murine fibroblasts (3T3) were suspended in hydrogels in rectangular capillary tubes exposed to fresh culture medium at one end and closed at the other to create an oxygen gradient (top image of Figure 1). Next, a hydrogel plug was cast into a custom bioreactor as shown in Figure 2. Widefield fluorescence microscopy and image processing were used to remove imaging artifacts such as uneven spatial illumination intensity and account for non-uniform sensor distribution. Metabolised Alamar Blue reagent was perfused (through) or convected (around) agarose hydrogels to demonstrate the establishment of a fluorescent probe diffusion gradient. Slow (.2mL/min) and fast (5mL/min) flow rates were assessed. Imaging was performed to quantify the time required for uniform distribution.

Results:

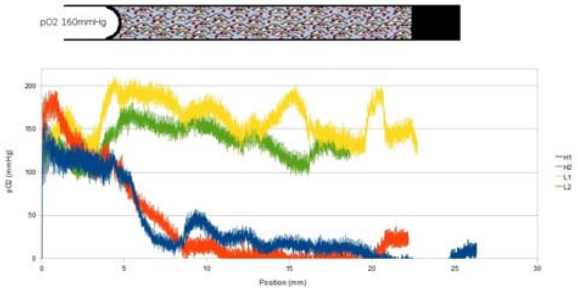

Figure 1. Oxygen gradient measurement by dissolved oxygen sensors within a capillary tube.

Fluorescence imaging of cellular hydrogels in capillary tubes demonstrated the establishment of an oxygen gradient as expected, from a high value at the oxygenated medium to a low value deeper within the hydrogel. Absolute estimates of pO_2 (Fig. 1) were calculated from atmospheric oxygen (acellular) and zero oxygen controls. L1 and L2 (green/yellow), i.e. low cell density samples ($5.5x10^5$ cells/mL), show no oxygen gradient, whereas a steep gradient is seen in high density samples H1, H2 (red/blue, $1x10^7$ cells/mL).

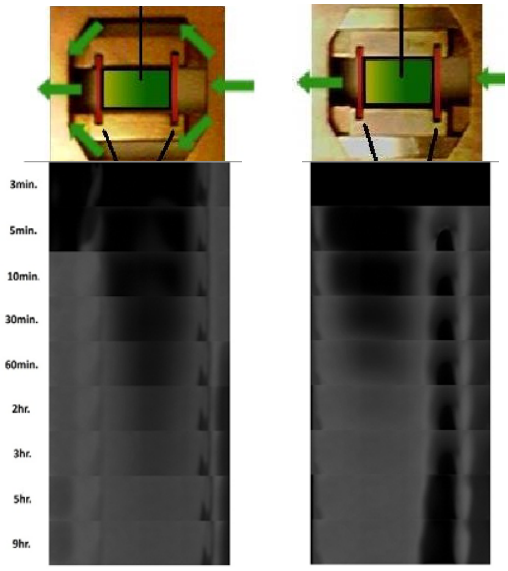

Figure 2. Hydrogel (green block) is cast between the two plugs in the custom bioreactor, which are replaced with permeable mesh inserts (red vertical bars). Channels remain open for convective flow (left), channels are blocked for perfusion flow (right). The series of images show the evolution of oxygen concentration distribution over 9 hours at flow rate of 0.2mL/min, where white indicates higher oxygen concentration.

The bioreactor results indicate that the times required for uniform Alamar Blue distribution were >9 hours and 5 hours for convective and perfusion flow (Fig. 2) at 0.2mL/min respectively, and 5 hours for convective flow at 5.0mL/min (perfusive sample destroyed at high flow rate).

Conclusions: Image processing techniques successfully eliminated artifacts (uneven illumination from mercury fluorescence lamp) and allow software stitching of images to facilitate quantitative fluorescence imaging over large sample areas. The custom-built bioreactor creates a one-dimensional molecule gradient through a hydrogel and can be used for future hypoxia and nutrient gradient testing of tissue engineered scaffolds. For example, data suggest that Alamar Blue may be used for spatial cell metabolic measurement if given sufficient time for uniform distribution. Future work will include testing of long-term cell viability in the custom bioreactor and quantitative, non-destructive oxygen measurements on large-scale samples.

References:

1. MH Sheridan et al., *J Control Release* 64:91–102 (2000).
2. K Kellner et al., *Biotechnol Bioeng* 80:73–83 (2002).
4. C Wu et al., *J Angew Chem Int Ed Engl* 48(15):2741-5 (2009).

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