Development of Probe Free Mapping of Cell Viability in Hydrogels

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Statement of Purpose:
Diffusion of oxygen in the interior of cell-encapsulated tissue scaffolds is of critical importance to cell viability in constructs of clinically relevant sizes [1]. It has been shown that depletion of oxygen within the scaffold leads to a decrease in cell density and viability [2]. Non-destructive methods that use fluorophore labeling to assess cell viability are also susceptible to problems with probe diffusion to the scaffold interior. Here, we seek to develop a method for *in vitro* spatial mapping of cell viability without using fluorescent labeling. The goal of this work is to establish contrast mechanisms for distinguishing between live and dead cells using a high sensitivity technique called optical coherence microscopy.

Methods:
Cell Culture and Encapsulation
To encapsulate L-929 mouse fibroblasts in 3D collagen gels, 5 x 10^4 cells/mL were suspended in a solution prepared by mixing 1 part of 10X minimum essential medium (MEM) with 8 parts of 3 mg/mL bovine collagen type I (Purcol, Advanced Biomatrix) with pH adjusted to 7.4 using NaOH, and allowed to set at 37°C for 2 h. Growth medium prepared from MEM (Eagle) with 10 volume % fetal bovine serum containing 2 mmol/L L-glutamine and 1mmol/L sodium pyruvate. Gels and cells were cultured for 2 d prior to imaging. In some cases, sodium azide (0.05 mass/volume %) was added to kill cells 12 h before imaging.

Optical Coherence Microscopy
The custom spectral domain optical coherence microscope (OCM), similar to a previously described instrument [3], uses interferometry for detection of returning light with very low power, as low as 20 μW. This approach enables non-invasive imaging of features that closely match the refractive index of their surrounding medium, such as live cells in a hydrogel. A 0.95 NA water immersion objective was used for imaging of the cells and collagen in media.

Results:
Figure 1 displays representative images of cells without (A, B, C) and with (D, E, F) azide treatment. Figure 1A is a phase contrast image of live L929 cells in collagen gel. The cells are not easily seen because of index matching with the surrounding collagen. A representative OCM image of a single live cell is shown in Figure 1B. The shape of the cell is not readily identified from the scattering of the collagen background. However, when successive images are collected, the subcellular motion generates scattering at different locations within the cell. Figure 1C is a composite of four consecutive OCM images collected over 3 min constructed from a logical "or" operation between each image. Following composite processing, the live cell can be better seen in the center of the image. The dead L929 cells have different optical properties than their live counterparts. The phase contrast image of the cell in collagen exposed to sodium azide is shown in Figure 1D. The L929 cells show an increase in contrast that results from an increase in scattering from the subcellular components of the dead cells. A representative OCM image of a single dead cell is shown in Figure 1E. Because the dead cell exhibits higher scattering than the live cell, the collagen background is suppressed and the cell morphology is more easily seen. The composite image in Figure 1F shows a well defined morphology with subcellular features. For this system, differences in optical scattering provide the contrast mechanism by which live versus dead cells can be identified *in situ*.

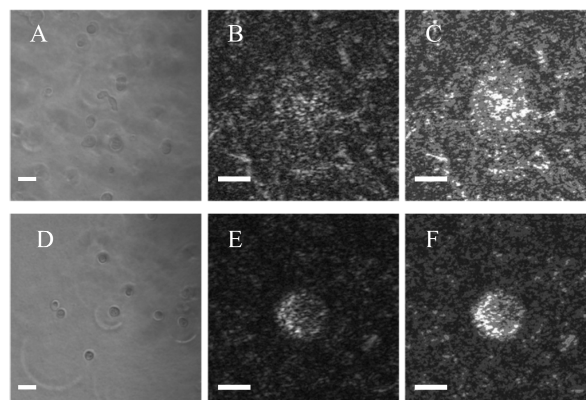

Figure 1: Images of untreated (A,B,C) and azide treated (D, E, F) L929 cells in collagen. Phase contrast (A, D), single OCM image (B, E), composite of four OCM images (C,F). Scale: 10 μm.

Conclusions: These results demonstrate the potential for OCM to noninvasively measure cell viability in 3D gel scaffolds without requiring fluorescent staining. Scaffolds can be returned to the incubator after imaging for continued culture eliminating the need for end-point cell testing. Future work will focus on corroborating live/dead assignments by comparing OCM with results from traditional fluorescent probes for viability.

References:
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2. Volkmer, E. et al., Tissue Eng Part A. 2008; 14: 1331-1340.
3. Xu, CY. et al., Opt. Lett. 2006; 31: 1079-1081.

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