Identifying Regions of Hypoxic Signaling within Three-Dimensional Stem Cell Aggregates for Engineering Cartilage

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Statement of Purpose: Tissue engineering is a recent approach for repair or replacement of compromised tissues, especially those with poor native regenerative capacity such as cartilage. Numerous current studies report attempts to engineer replacement cartilage by selectively encouraging chondrogenesis in populations of mesenchymal or adipose-derived stem cells. These cells are often aggregated and/or seeded into a 3-D scaffold and then driven to differentiation by introduction of select biochemical stimuli such as growth factors. However, low oxygen tension is also an important promoter of the chondrogenic phenotype, as cartilage exists in hypoxic sites, *in vivo*. Still, current studies show very little emphasis on incorporating both biochemical and hypoxic stimuli to encourage cartilage formation. Those studies that do, simply apply a universal hypoxic condition without examining how 3-D geometry affects the oxygenation of individual cells. The purpose of the current research is to identify how three-dimensional geometry contributes to the development of hypoxic gradients within tissue aggregates and scaffolds. Such an understanding will be important to improving cartilage engineering techniques as well as other biological research in which low oxygen tension plays a key role.

Methods: Adipose-derived stem cells (ADSCs) were genetically engineered using an in-house adenovirus system. Infected cells received a gene coding for the red fluorescent protein, DS-Red, under control of a minimal SV40 promoter and three copies of the hypoxia-responsive element (HRE) enhancer. In normoxia, the transcription factor, hypoxia-inducible factor-1α (HIF-1α), is expressed but rapidly degraded. When oxygen concentrations within the cell drop, HIF-1α is stabilized and dimerizes with HIF-1β to form a unit that binds to the HRE and initiates transcription of the downstream gene. Thus, hypoxic cell produce DS-Red and fluoresce. Apart from being the primary regulator of cellular hypoxic responses, HIF-1α has been implicated as being important to the process of chondrogenesis, making it an extremely relevant hypoxia marker target.

ADSCs were infected with the marker and aggregated to different sizes, then placed in incubation at either 20% or 1% O_2. Aggregates were then periodically imaged on an inverted fluorescent microscope. Uninfected ADSCs were likewise aggregated and subjected to the same oxygen conditions. Aggregates were then periodically fixed, immunofluorescently stained for HIF-1α, and imaged. Imaging software was used to create signal intensity profiles for both the hypoxia marker signal and the HIF-1α staining signal.

Results: Regions of hypoxic stress within the aggregates were successfully indicated by the hypoxia marker system and showed good similarity to expression of HIF-1α. Figure 1 (top) shows fluorescent hypoxia marker signal in an aggregate incubated in 1% O_2 for 2 days, with an

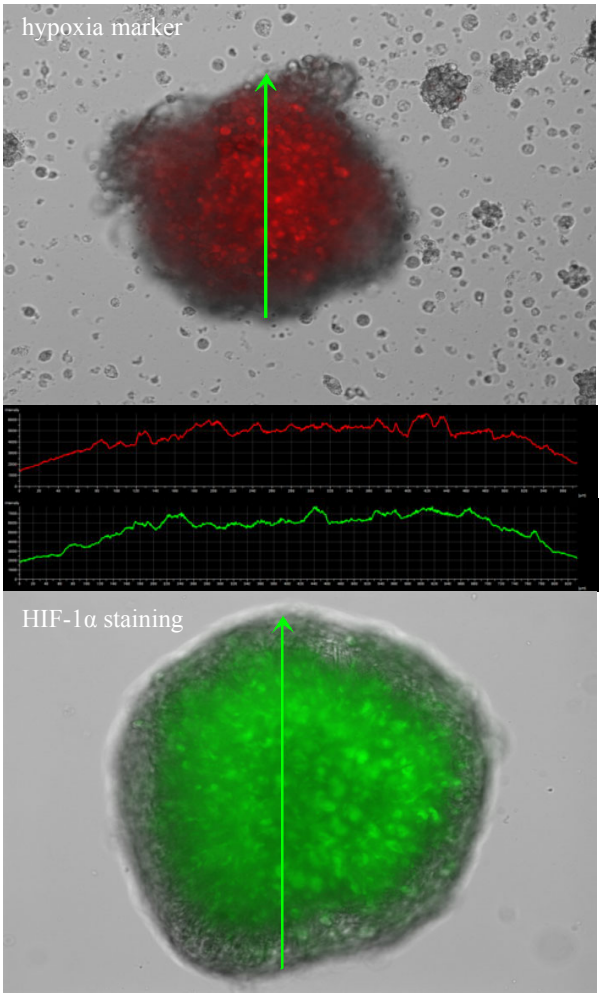

Figure 1.

intensity profile of the fluorescence beneath. The bottom image shows immunofluorescent staining for HIF-1α in an aggregate also incubated in 1% O_2 for 2 days, accompanied by its intensity profile. Smaller aggregates incubated in 20% O_2 did not express the hypoxic signal, though large aggregates displayed central signaling. Observation of the signal intensity profiles indicate more severe hypoxia response occurring in interior aggregate regions with less hypoxic indication on the periphery.

Conclusions: These results clearly identify the appearance of hypoxia gradients within large, 3-D tissue aggregates. This regionalization may play an important role in tissue engineering outcomes in applications where hypoxic stress is an important driving event, as this stress is clearly experienced unequally by different cells in the aggregate. Future studies will examine the timing of hypoxia signaling onset, differences in regional hypoxia signal expression in aggregates of different sizes, and viability of cells within aggregates of different sizes in both high and low oxygen conditions.