Controlling Secretion of VEGF from 3D Stem Cell Aggregates to Enhance Osteochondral Bone Regeneration

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Statement of Purpose: The ability of adipose-derived stem cells (ADSCs) to produce and secrete pro-angiogenic factors, such as vascular endothelial growth factor (VEGF), has recently been reported. In addition to its potent mitogenic effect on endothelial cells, VEGF is also an important survival and maturation factor for chondrocytes during osteochondral bone formation/repair allowing for the formation of a cartilaginous template that is later ossified. Thus, ADSCs may represent a promising cell-based source for the delivery of VEGF to enhance the regeneration/engineering of bone tissue, offering certain advantages over material-based delivery techniques. For the approach to be effective, however, it will be necessary to be able to control VEGF secretion in a reliable manner. Low oxygen tension (hypoxia) is known to upregulate VEGF production. Therefore, we investigate how regulation of hypoxic status through 3D culture and manipulation of external oxygen concentration can impact the secretion of VEGF from ADSCs.

Methods: Using a viral vector, ADSCs were modified to express a fluorescent reporter protein only when hypoxia-inducible factor-1 (HIF-1) was sufficiently stabilized as a result of low intracellular oxygen concentrations. ADSCs were grown as monolayers or formed into spheroids containing 5,000 (5k); 10,000 (10k); 20,000 (20k); or 60,000 (60k) cells by centrifugal pelleting. Monolayers and spheroids of each size were cultured in 20%, 2%, or 1% O_2 and imaged daily. Daily media samples were analyzed by ELISA to quantify vascular endothelial growth factor (VEGF) secretion. ADSC viability was assessed by membrane permeability/esterase activity and evaluation of hematoxylin and eosin stained histological sections.

Results: HIF-1 activity increased in a graded manner as external oxygen concentration decreased and as spheroid size increased (Figure 1).

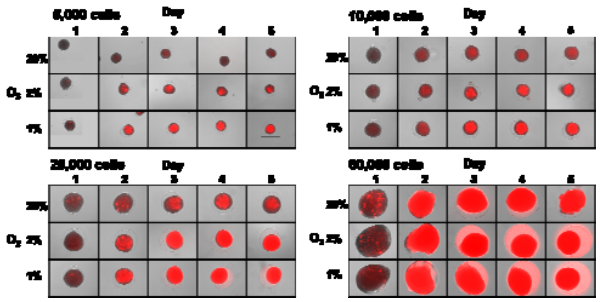

Figure 1. Fluorescent reporter of HIF activity in ADSCs

Correspondingly, ADSCs cultured in 2% and 1% O_2 secreted higher levels of VEGF than those cultured in 20% O_2. For all size groups, secretion was highest on Days 1 and 2 and declining thereafter, though the difference was less pronounced in 20k and 60k spheroids. In 2% and 1% O_2, per-cell VEGF secretion was highest in 10k spheroids, even though 20k and 60k spheroids displayed greater HIF activity (Figure 2).

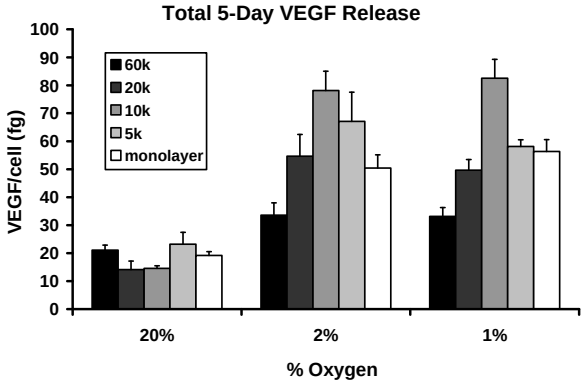

Figure 2. Cumulative VEGF secretion from ADSCs

Membrane permeability/esterase activity assays showed high viability in all samples, however, histology revealed signs of apoptosis/necrosis in the cores of 20k and 60k spheroids cultured in 2% and 1% O_2, which likely accounts for their reduced secretion efficiency (Figure 3).

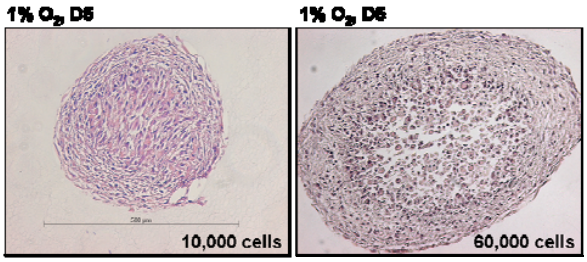

Figure 3. Histological sections of 10k and 60k spheroids

Conclusions: Hypoxic status of ADSCs can be easily monitored over time with the fluorescent reporter system and can be controlled by manipulating external oxygen concentration and tissue size. Manipulation of these two parameters also allows for control over secretion of VEGF, which can be optimized. Additionally, hypoxia and HIFs are known to be linked to enhanced chondrogenesis through VEGF-independent pathways. Thus, 3D cultures of ADSCs represent an appealing cell-based approach to promote cartilage formation during bone repair. Culture size and oxygenation should be carefully considered in order to achieve the optimal therapeutic result.

References:

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