Differentiation of Adipose Derived Stem Cells on Nanofibrous Collagen and Elastin Matrices

Michael Springer, M.S., Josephine Allen, Ph.D.
University of Florida, Department of Materials Science and Engineering

Statement of Purpose: Electrospun collagen and elastin matrices have gained widespread interest in the scientific community as a promising option for tissue engineering. The high porosity, high surface area, and natural composition of these electrospun scaffolds are ideal for vascular tissue engineering. In the same regard, the morphology of these scaffolds mimics the fibrous nature of arterial walls. It is well known that adult stem cells have a role in vascular remodeling *in vivo* and are a promising cell source for tissue engineering applications. The differentiation of adult stem cells is influenced by the multiple factors such as extracellular matrix proteins, soluble growth factors, and cytoskeletal arrangement. The focus of this project is to investigate a strategy to differentiate adipose derived stem cells (ADSCs) on three dimensional protein scaffolds to create a functional medial layer for tissue engineered small diameter arteries.
Methods: ADSCs were cultured on fibers of three different protein compositions: 100% collagen, 100% elastin, and 80% collagen with 20% elastin. In order to obtain electrospun fibers, collagen type I from calf skin and elastin from bovine neck ligament (Elastin Products) were each dissolved in 1,1,1,6,6,6-hexafluoroisopropanol (HFIP) at a concentration of 12% and 20% (w/v) respectively. For the mixed fibers, collagen and elastin were combined in HFIP at a concentration of 6.64% and 1.66% respectively. Electrospinning conditions were set to obtain uniform fibers for each protein composition. Average diameter of the scaffolds was 2.43 ± 0.9µm. Following, ADSCs (Lonza) were cultured onto the scaffolds in Dulbecco's Modified Eagle Medium/Ham's F12 (DMEM/F12). TGF-β (1ng/mL) was added to half of the samples to promote differentiation of ADSCs into smooth muscle cells. For controls, ADSCs were also cultured on tissue culture plastic (TCP) with and without TGF-β supplementation. After 14 days of culture, the cells were lysed, RNA was isolated, and Reverse Transcription-quantitative Polymerase Chain Reaction (RT-qPCR) analysis was performed. Myosin Heavy Chain (MYH11) and α-Actin (ACTA2) gene expression were both analyzed relative to β-Actin.
Results: Gene expression of both ACTA2 and MYH11 was significantly upregulated for ADSCs when supplemented with 1ng/mL TGF-β on TCP (Figure 1). Furthermore, there was a significant increase of α-actin expression of all samples of ADSCs on electrospun fibers regardless of protein composition (Figure 2). The additional supplementation of TGF-β to ADSCs cultured on fibers did not significantly impact ACTA2 expression compared to fibers without TGF-β (data not shown).
Conclusions: Our results suggest that a 14 day culture of ADSCs supplemented with 1ng/mL TGF-β is sufficient to induce smooth muscle cell differentiation. The increase in smooth muscle myosin heavy chain expression that was observed indicates that the ADSCs are differentiating into smooth muscle cells as myosin heavy chain is specific to mature smooth muscle cells and is only expressed in the later stages of differentiation[1]. These results agree with what has been previously found when culturing mesenchymal stem cells with TGF-β[2].

ADSCs cultured on protein fibers all show significant increases in smooth muscle cell gene expression. Under all conditions (with and without TGF-β), smooth muscle α-actin expression showed significant upregulation. Stimulation with TGF-β did show small increases in α-actin expression when compared to non-supplemented samples; though this difference was not significant. Expression of α-actin was increased with the increasing concentration of elastin in the electrospun scaffolds. This suggests that the contribution of elastin toward the differentiation of ADSCs into SMCs is more significant than that of collagen.

Lastly, the temporal changes in gene expression by differentiating ADSCs when cultured on electrospun protein fibers and overall functionality are being investigated.

References:

1. Owens GK. Physiol Rev. 1995;75:(3) 487-517
2. Gong Z. FASEB J. 2008;22:(6) 1635-1648

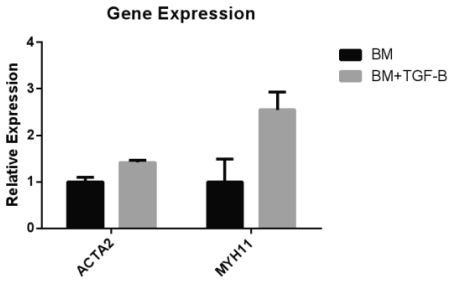

Figure 1 RT-qPCR analysis of ADSCs cultured in DMEM/F12 basal medium (BM) and DMEM/F12 supplemented with 1ng/mL TGF-β (BM+TGF-B)

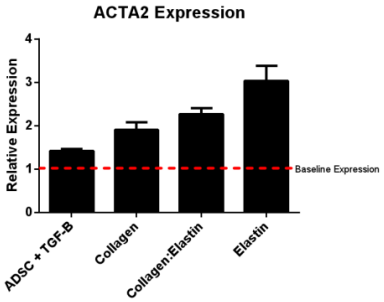

Figure 2 RT-qPCR analysis of ADSCs cultured on electrospun fibers in DMEM/F12 without TGF-β.