Vascular Smooth Muscle Cell Alpha-Smooth Muscle Actin Expression after Exposure to Conditioned Media from Endothelial Cells Cultured in Oscillatory Flow Environments

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Introduction: The vascular wall consists of a layer of vascular endothelial cells (VasEC) and a sublayer of vascular smooth muscle cells (VasSMC). Vascular remodeling often involves paracrine signaling between VasECs and VasSMCs, and diseases such as atherosclerosis can result from improper communication between these cells. Atherosclerosis also associates with regions of oscillatory flow at arterial branch points, where altered VasEC-VasSMC signaling may initiate remodeling responses [1]. Recent mechanistic studies have utilized steady versus purely oscillatory flow patterns; however, the degree of oscillations has also been shown to affect cell phenotype [2]. Alpha smooth muscle actin (αSMA) is a protein that regulates contractility of VasSMCs, and changes in expression are critical to pathology of early atherosclerosis [3]. In this study, we specifically examined the relation between levels of αSMA expression in VasSMCs after exposure to conditioned media obtained from VasECs exposed to various flow oscillations.

Materials and Methods: Porcine VasECs were seeded for 24 hours at 2 x 10^5 cells per channel in 24-well Bioflux plates consisting of 8 microfluidic channels per plate (Fluxion Biosciences, San Francisco, CA). VasECs were then conditioned for 48 hours at a magnitude of 1 dyne/cm^2, and static cultures were used as a baseline control. Using oscillatory shear index (OSI; equation 1) as a parameter that quantifies change in direction of wall shear stresses [4], the following OSIs were applied to the VasECs: Static (control), steady flow (OSI=0), 0.25 OSI, and 0.50 OSI.

$$OSI = \frac{1}{2}\left(1 - \frac{\left|\int_0^T \tau_\omega dt\right|}{\int_0^T |\tau_\omega| dt}\right)$$ *Equation 1*, where τ_ω = wall shear stress, T = duration of cycle, t = time.

The conditioned media from VasECs from each flow group were subsequently used to culture VasSMCs for 48 hours to simulate paracrine regulation between VasECs and VasSMCs. VasSMC expression of αSMA were assessed using RT-qPCR.

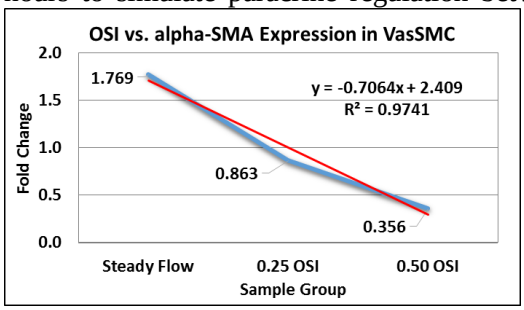

Figure 1. Relation between OSI and aSMA from VasSMC cultured in flow conditioned VasEC media.

Results and Discussion: Highest expression of αSMA was observed in VasSMCs (n=6) exposed to steady flow conditioned media from endothelial cells (OSI=0; Fig. 1). Expression of αSMA decreased linearly (R^2=0.97) with an increase in oscillatory flow magnitudes, reaching a minimum expression at the maximum possible OSI value of 0.5. Statistical analysis showed a significantly higher expression ($p<0.05$) of αSMA in the steady flow group (OSI=0) compared to OSI=0.50. On the other hand, comparisons of αSMA gene expression between the other flow groups (OSI=0 vs. OSI=0.25 and OSI=0.25 vs. OSI=0.5) were not significant ($p>0.05$). This finding suggests that VasECs exposed to moderate levels of flow oscillations will, via paracrine signaling, maintain the contractility phenotype of VasSMCs in a similar manner to VasECs exposed to steady flow.

Conclusions: Downregulation of αSMA expression by VasSMCs with increasing VasEC-exposed, OSI-states suggests loss of a contractile, non-proliferative phenotype [5]. Contrary to current theories, very high exposure rather non-exposure of VasECs to oscillatory flow environments appears to induce the progressive reduction in VasSMC αSMA gene expression; however, whether this loss is a result of phenotypic switching to a pro-inflammatory phenotype leading to vascular intimal thickening and subsequently, atherosclerosis, needs to be further investigated.

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References:

[1] Dunn, J., Thabet, S., & Jo, H. (2015). Flow-Dependent Epigenetic DNA Methylation in Endothelial Gene Expression and Atherosclerosis. *Arteriosclerosis, thrombosis, and vascular biology*, *35*(7), 1562–1569. doi:10.1161/ATVBAHA.115.305042
[2] Rath S, et al. Differentiation and Distribution of Marrow Stem Cells in Flex-Flow Environments Demonstrate Support of the Valvular Phenotype. PloS One 2015;10(11):e0141802.
[3] Allahverdian, S. et al. (2018). Smooth Muscle Cell Fate and Plasticity in Atherosclerosis. Cardiovascular research. 114.
[4] He X, Ku DN. Pulsatile Flow in the Human Left Coronary Artery Bifurcation: Average Conditions. ASME. *J Biomech Eng.* 1996;118(1):74-82.
[5] Chen, L et al. (2016). Smooth Muscle-Alpha Actin Inhibits Vascular Smooth Muscle Cell Proliferation and Migration by Inhibiting Rac1 Activity. *PloS one*, *11*(5), e0155726. doi:10.1371/journal.pone.0155726