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THE EFFECTS OF OSCILLATORY SHEAR REGULATION ON PARACRINE SIGNALING BETWEEN VASCULAR ENDOTHELIAL CELLS AND VASCULAR SMOOTH MUSCLE CELLS

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INTRODUCTION

The vascular wall consists of a layer of endothelial cells (VasEC) that are directly in contact with blood flow, 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.

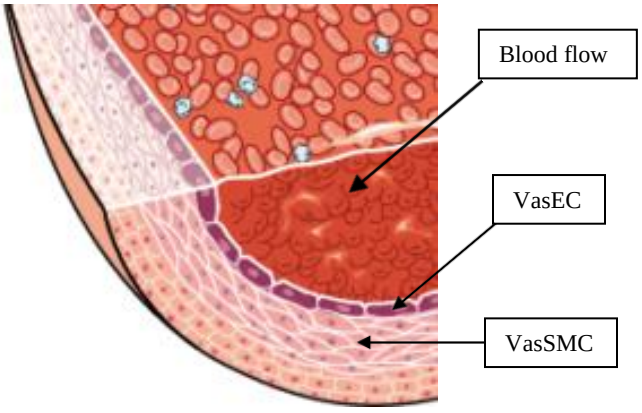

Figure 1: Vascular Endothelial Cells (VasECs) and Vascular Smooth Muscle Cells (VasSMCs)

VasECs are known to respond to hemodynamic stimuli, and VasEC dysfunction in atherosclerosis occurs preferentially at regions exposed to oscillatory flow. Studies have demonstrated development of cardiovascular tissues under dynamic environments such as flow, flexure, and stretch, in which have significant impact on cell phenotype [1]. In this regard, there may be a range of oscillations that maintains vascular tissue integrity. In the current study, we conditioned VasECs under various specific oscillatory flow profiles, which can be quantified by the oscillatory shear index (OSI) parameter. It is widely accepted that biomechanical cues are critical in maintaining vascular tissue homeostasis; however, oscillation dependent changes in cell communication and molecular regulation has not been thoroughly investigated. We therefore examined the paracrine signaling of biochemical end-products between VasEC and its sublayer, VasSMC, through the biochemical environment resulting from physiologically relevant oscillations.

METHODS

Oscillatory shear index (OSI; Equation 1) is a parameter that quantifies the change in direction and magnitude of wall shear stresses [2]. The following OSI magnitudes were applied to the VasECs: static (no flow), steady flow (OSI = 0), 0.25 OSI, and 0.5 OSI.

OSI is defined as:

$$OSI = \frac{1}{2}\left(1 - \frac{\left|\int_0^T \tau_\omega dt\right|}{\int_0^T |\tau_\omega| dt}\right) \quad (1)$$

Where τ_ω = wall shear stress, T = duration of cycle, t = time

VasECs were seeded for 24 hours at 2.0 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 later

conditioned for 48 hours using a shear stress cell assay system (Fluxion Biosciences) at a magnitude of 1 dyne/cm^2 [3].

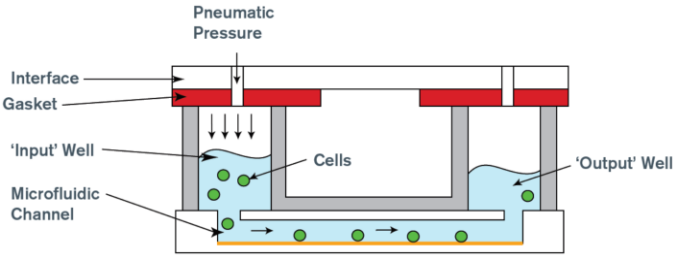

Figure 2: Schematic of microfluidic channels

The conditioned media from VasECs for each flow group were collected. These media were subsequently used to culture VasSMCs for 48 hours using 8 x 10^5 cells in 6-cm dishes. After exposure to the conditioned media of the various flow groups, key phenotypic markers expressed by VasSMC were assessed using RT-qPCR at three replicates per target gene per flow sample group. Data from RT-qPCR consisted of cycle threshold, or C_T values, which were analyzed using the Livak method, $\Delta\Delta C_T$ [4], to compute fold change with the static (no flow) sample group as control.

RESULTS

Three VasSMC samples (n=3) at three replicates per target gene were analyzed. Higher expression of smooth muscle alpha-actin (alpha-SMA) was observed in VasSMCs exposed to non-static VasEC conditioned media compared to those exposed to the static media (Figure 3). Alpha-SMA expression was highest in VasSMCs exposed to steady flow (OSI = 0). Compared to the steady flow group, alpha-SMA expression decreased with increasing levels of OSI.

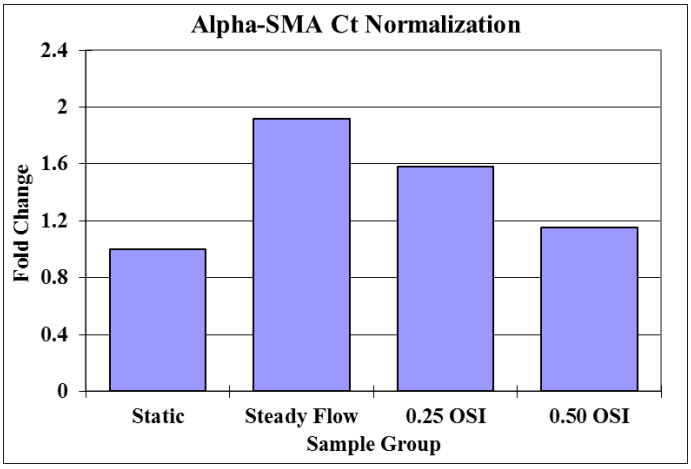

Figure 3: Expression of Alpha-SMA from Vascular Smooth Muscle Cells

DISCUSSION

We observed that various oscillatory flow profiles alter cell responses to its immediate environment via both cell-to-cell paracrine and autocrine communication. A higher expression of alpha-SMA from VasSMC in non-static groups of conditioned media may indicate that fluid motion promotes paracrine signaling for fibrotic development. As alpha-SMA is a marker for contractile phenotype of adult smooth muscle cells, non-static flow groups experienced by VasEC may have led to the secretin of factors then enabled paracrine regulation of VasSMC phenotype.

It is known that VasSMCs exhibit a less proliferative and more contractile phenotype with abundant alpha-SMA [5]. The VasSMCs exposed to steady flow conditioned VasEC media expressed the highest level of alpha-SMA amongst all sample groups. As oscillatory flow was introduced to the system, the expression of alpha-SMA decreased. This supports previous observations of vascular remodeling in regions of oscillatory flow.

Previous studies have shown the role of disturbed flow in VasEC physiology and pathogenesis of vascular diseases. However, in this study, we have further verified that through OSI-initiated paracrine signaling, the biochemical end-products released from VasECs under disturbed flow is also transmitted to the sublayer cells, or the VaSMCs, thereby potentially affecting their phenotype.

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