Calcific Media Combined with Media from Oscillatory Flow-Conditioned Valve Endothelial Cells Leads to Valve Interstitial Cell Calcification

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Introduction: The aortic valve facilitates unidirectional blood flow from the heart to the aorta for systemic blood distribution. Aortic valve biomechanical function relies on the action of thin, membranous leaflets that open and close the valve orifice over the course of the cardiac cycle. A monolayer of valve endothelial cells (VECs) reside on the outer surface of the aortic valve leaflets. Underneath the VECs is a sublayer of valve interstitial cells (VICs). Valvular remodeling often involves paracrine signaling between VECs and VICs, and diseases such as valve calcification can result from improper communication between these cells. VECs are known to respond to hemodynamic stimuli, and studies have shown that VECs exposed to disturbed flow can result in pro-inflammatory phenotypic changes and endothelial-mesenchymal-transition [1]. Valve endothelial dysfunction can then lead to phenotypic switching of quiescent VICs to osteogenic VICs, resulting in valve calcification. However, the effect of valve calcification due to alterations in fluid oscillations remains unclear. In the present investigation, we used oscillatory shear index (OSI) as a parameter to quantify the change in shear stress direction. OSI ranges from 0 (no oscillations) to 0.5 (equal forward and reverse fluid flow) [2]. We examined VIC calcification in response to equal amounts of pro-calcific media (PM) and conditioned media from VECs cultured under different OSI flow environments.

Materials and Methods: Rat VECs were purchased from Celprogen, Inc. (Torrance, CA) and expanded in T75 flasks. VECs were seeded for 24 hours at $2.0x10^5$ cells per channel in 24-well Bioflux plates consisting of 8 microfluidic channels per plate (Fluxion Biosciences, Inc. Alameda, CA). The VECs were conditioned for 48 hours in the Bioflux shear stress cell assay system at an average shear stress magnitude of 1 dyne/cm^2 under the following OSIs: static (0 OSI/no flow), steady flow (0 OSI/steady flow), 0.25 OSI (moderate oscillation), and 0.50 OSI (full oscillation). Rat VICs were purchased from Innoprot (Bizkaia, Spain) and expanded in T75 flasks. The conditioned media from VEC groups were collected and subsequently used to culture VICs in 12-well plates with equal volume of PM [3, 4]. VIC conditioning lasted for 7 days with one media change on day 4. Upon termination of VIC exposure to various VEC flow group media, VIC calcification was measured by Alizarin Red staining. The Alizarin Red dye was then extracted and quantified with a microplate reader. N=3 biological replicates were conducted for each VIC conditioning group. Data was evaluated using 1-way ANOVA in SPSS with statistical significance at $p<0.05$.

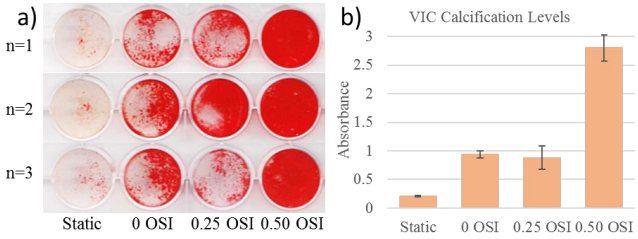

Figure 1: a) Alizarin Red staining (ARS) of VIC in oscillatory flow-conditioned VEC media with pro-calcifying components. b) Absorbance at 405nm after ARS dye extraction with 10% (v/v) CH_3COOH and 10% (v/v) NH_4OH. Plot data are expressed as mean (n=3) with error bars representing standard error of the mean.

Results and Discussion: The highest VIC calcification was observed in the 0.50 OSI group. Specifically, statistical assessment showed significantly increased calcification ($p<0.001$) in the 0.50 OSI group compared to the static, 0 OSI (steady flow), and 0.25 OSI groups. Comparisons of VIC calcification between 0.25 OSI vs. Static and 0.25 OSI vs. 0 OSI (steady flow) were found to be not significant ($p>0.05$). This finding suggests that VECs exposed to low-to-moderate levels of flow oscillations maintain a quiescent VIC phenotype via paracrine signaling. On the other hand, augmented extracellular matrix calcific conditions coupled with high oscillatory flow regions (OSI=0.50) on VECs, leads to substantial risk of increasing VIC calcification.

Conclusions: Combination of pro-calcific media with high OSI (OSI=0.50) significantly increases ($p<0.05$) VIC calcification via VEC paracrine signaling. This corroborates current theories of increased VIC calcification under disturbed flow [1] and the fact that calcification occurs on the aortic aspect of valve leaflets, where flow oscillations are observed. However, whether molecular targets in this VEC-to-VIC paracrine regulated pathway can be targeted to reduce valve calcification needs to be further investigated.

References:

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