Title: Valve Endothelial Cells Exposure to High Oscillatory Flow Leads to Valve Interstitial Cell Calcification

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Objective:
Valve remodeling involves paracrine signaling between VECs and VICs under hemodynamics. In this study, we used oscillatory shear index (OSI) to quantify temporal changes in fluid-induced shear stress direction. OSI ranges from 0 (no oscillation) to 0.5 (full oscillation) [1]. We examined VIC calcification response to conditioned media from VECs under different OSIs.

Methods:
Human VECs (LonzaBioscience) and VICs (Innoprot) were expanded in culture. VECs were seeded for 24hrs at 20000 cells per microfluidic channel in Bioflux plates (FluxionBiosciences) and conditioned for 48hrs in a shear assay system at 1 dyne/cm^2 under the following OSIs: static, steady flow, 0.25OSI, and 0.50OSI. Conditioned media from VEC groups were collected, and a portion was ultracentrifuged at 50,000RPM. The non-exosomal supernatants removed and the exosome pellets were resuspended in fresh media. Original VEC-conditioned media and the ultracentrifuged non-exosomal and exosome groups were then used to culture VICs (n=3/group) with equal concentrations of pro-calcifying (PC) ingredients: 1.8mM $CaCl_2$, 3.8mM NaH_2PO_4, and 0.4units/mL of inorganic pyrophosphate [2,3] at 5% FBS and 1% P/S. Fresh PC was used as control. VIC culture lasted 7 days, followed by alizarin red staining and quantification. Data was normalized to protein level and ANOVA statistical analyses was performed.

Results:

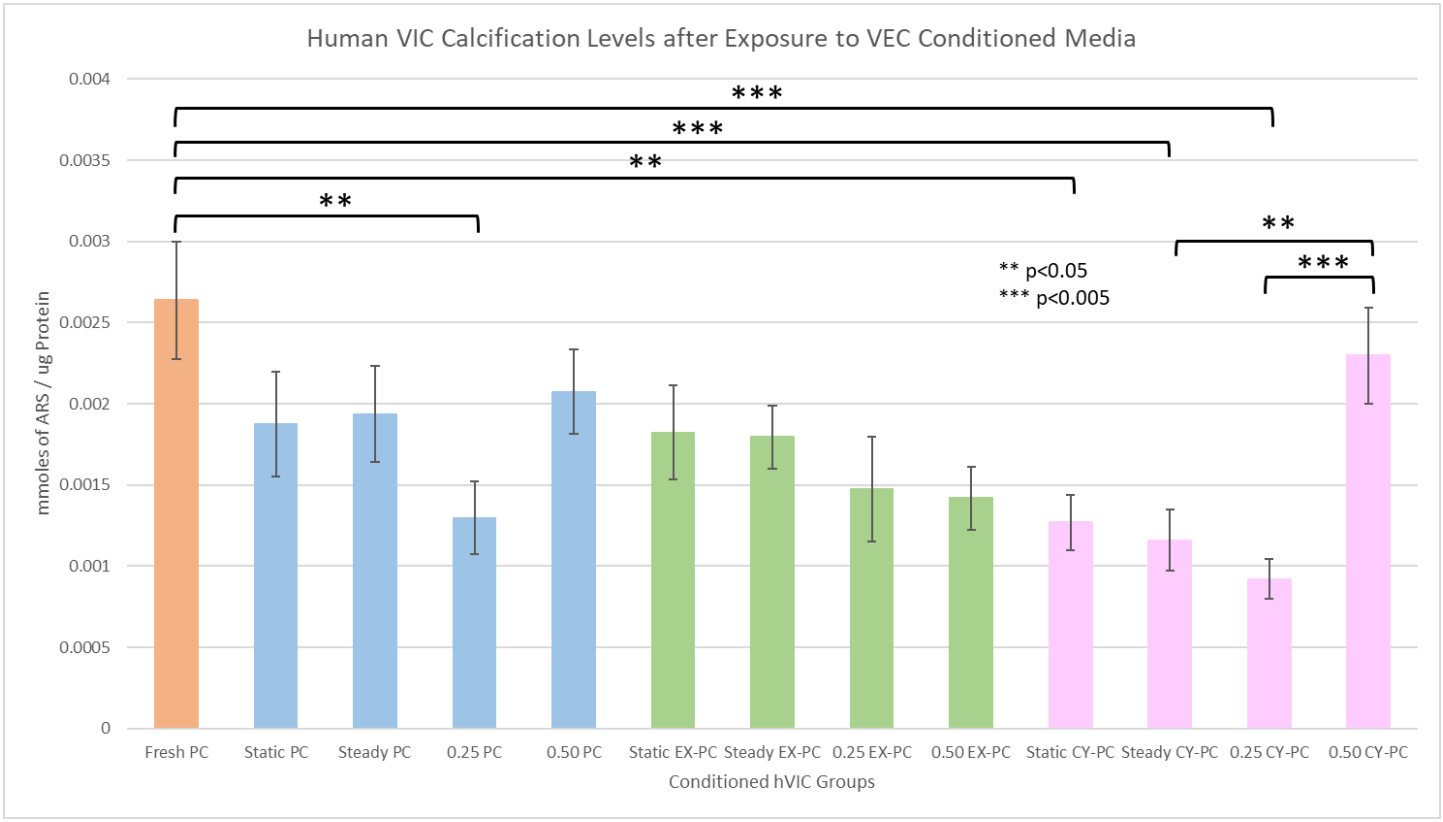

Figure 1: Alizarin red quantification per amount of protein in each group. PC group contains pro-calcifying ingredients. EX group corresponds to the exosomal pellet after ultracentrifugation while CY group corresponds to the non-exosomal cytokine supernatant after ultracentrifugation.

Conclusions:
Significant VIC calcification was observed in high OSI non-exosomal group (0.50 CY-PC). This suggests that non-exosomal cytokines released by the VECs are primarily responsible for inducing VIC calcification under biomechanically-induced high OSIs in combination with pro-calcific environments.

References:
[1] He&Ku.(1996).
[2] Rathan et al.(2014).
[3] Goto et al.(2019).