Chondroitin Sulfate Promotes Calcification in Aortic Valve Interstitial Cells

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Introduction:

Endothelial to Mesenchymal Transformation (EndMT) is a source of activated fibroblasts. Glycosaminoglycan (GAG) migration from the aortic valve spongiosa to the normally collagen-rich fibrosa has been shown to be a step in aortic valve disease progression. Our data suggests that the presence of GAGs in the extracellular matrix (ECM) promotes EndMT and contributes to the calcification process.

Materials and Methods:

To mimic the healthy and diseased aortic valve *in vitro*, Porcine Aortic Valve Interstitial Cells (PAVIC) were seeded into 3D 1.5 mg/mL collagen type I gels, 2.2 mg/mL collagen gels and 1.5 mg/mL collagen gels containing 20 mg/mL chondroitin sulfate (CS) at $1x10^6$ cells/mL. Porcine Aortic Valve Endothelial Cells (PAVEC) were seeded on top of the same 3D hydrogels at 95,000 cells/cm^2 to form a 3D aortic valve co-culture more consistent with the *in vivo* microenvironment. 2.2 mg/mL collagen gels are a stiffness control for 1.5 mg/mL collagen gels + 20 mg/mL CS. The PAVIC and PAVEC cells were cultured in DMEM+10% FBS for 14 days. On day 14, cells were fixed with 4% paraformaldehyde or processed for RNA isolation. Fixed cells were stained with Alizarin Red to visualize calcified nodules. Fixed cells were also stained for α-SMA protein expression. ACTA2, Osteocalcin, RUNX2, and PECAM1 gene expression was analyzed with quantitative PCR. Finally, GAG and collagen production by cells and cell proliferation was measured via ELISA.

Results and Discussion:

Hydrogels containing CS and PAVIC cells grown in regular culture medium showed that there was a significant increase in calcified nodule formation, pro-calcification gene expression (Osteocalcin, RUNX2), and activated fibroblast protein expression (α-SMA) when compared with PAVIC grown in type I collagen-only gels. DNA quantification analysis showed a greater amount of PAVIC proliferation in 1.5 mg/mL collagen+20 mg/mL CS hydrogels. The addition of PAVEC to the valve model decreased calcified nodule formation, pro-calcific gene expression markers, and activated fibroblast markers.

Conclusions:

Our findings suggest that GAGs within the ECM both promote EndMT and play a role in calcified nodule formation. These findings can aid in elucidating the underlying mechanisms for calcific aortic valve disease pathogenesis. Future experiments will probe the roles of mechanical stresses and inflammatory cytokines on the formation of calcific nodules in the 3D aortic valve model.

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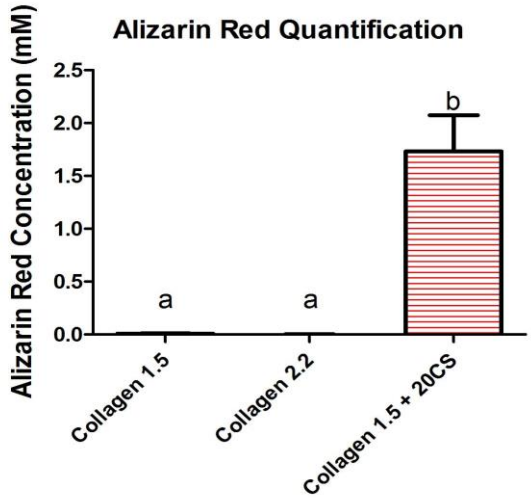

Figure 1. Alizarin Red absorbance in PAVIC grown for two weeks in DMEM+10% FBS. 3D hydrogels were composed of 1.5 mg/mL type I collagen (healthy valve condition), 2.2 mg/mL type I collagen (stiffness control), or 2.2 mg/mL type I collagen+20 mg/mL chondroitin sulfate (diseased valve condition). The presence of chondroitin sulfate in the 3D hydrogel resulted in a statistically significant increase in calcified nodule formation without osteogenic medium supplements. Standard error and Tukey groups are shown, $p < 0.05$, $n = 6$.