A three-dimensional co-culture model of the aortic valve using magnetic levitation

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Statement of Purpose: The aortic valve consists of valvular interstitial cells (VICs) and endothelial cells (VECs). While these cells are understood to work synergistically to maintain leaflet structure and valvular function, there are few co-culture models of these cell types. The lack of co-culture models of the aortic valve can partly be due to the inability to produce such models using traditional 2D cell culture techniques, with which the majority of research on valvular cells is conducted. 2D environments are poor representations of the native 3D environment in which valvular cells reside. Magnetic levitation is a recently discovered technique to create 3D cultures. These 3D cultures have been shown to increase cell proliferation and protein expression, and the technique can be used to construct co-cultures. In this study, 3D aortic valve co-cultures (AVCCs) were assembled using magnetic levitation and cultured for 3 days.

Methods: VICs and VECs were extracted from fresh porcine aortic valves obtained from a local abattoir, and cultured in an incubator until use. Magnetic levitation using the Bio-Assembler Kit (Nano3D Biosciences, Houston, TX) was employed to create 3D cultures. Cells were incubated with a nanoparticle assembly (NanoShuttle, Nano3D Biosciences) consisting of poly-L-lysine, magnetic iron oxide and gold nanoparticles that form a gel via electrostatic interactions that will render them magnetic and allows for their manipulation. To assemble the co-cultures, 500,000 cells of each type were levitated, and a magnetic pen was used to sequentially pick up the co-cultures (first VECs, then VICs) to create a co-culture with VEC and VIC layers. The co-cultures are referred to as the aortic valve co-culture (AVCC). The AVCC was then levitated in culture for 3 days.

The effects of both the NS and magnetic field on cell proliferation over 8 days were measured using an MTT assay. IHC was used to verify the maintenance of phenotype and function, and the formation of ECM. The antigens stained for in this study included: αSMA for VIC phenotype; CD31 for VEC phenotype; collagen type I (COL1), laminin (lam), and fibronectin (FN) for ECM; prolyl 4-hydroxylase for collagen synthesis; endothelial nitric oxide synthase (eNOS) and von Willebrand factor (VWF) for endothelial function; and VE-cadherin and N-cadherin for cell-cell interactions. qRT-PCR was performed (n=3-5) to measure the positive gene expression of: αSMA for VIC phenotype; CD31 for VEC phenotype; COL1A1, FN, Lam-β1 for ECM; lysyl oxidase (LOX) for collagen and elastin synthesis; and eNOS and VWF for endothelial function.

Results: Both VICs and VECs were successfully levitated into 3D cultures. Neither incubation with NanoShuttle nor exposure to the magnetic field significantly affected the proliferation of VICs and VECs. AVCCs were successfully assembled and maintained for 3 days. AVCCs stained positive for CD31 and αSMA, demonstrating phenotype was maintained (Fig. 1). αSMA gene expression in AVCC and 3D monotype cultures was significantly less αSMA mRNA than 2D VIC cultures. Functional markers eNOS, vWF, and P4H were present. Extracellular matrix components COL1, FN, and Lam also stained positive, with reduced gene expression of these proteins in 3D compared to 2D. Genes for COL1A1, LOX, and αSMA were expressed less in AVCCs than in 2D cultures, indicating that VICs are quiescent. eNOS and VWF gene expressions in 3D cultures v. 2D cultures suggest an anti-thrombotic nature. Co-localization of CD31 and αSMA in the AVCC suggest endothelial-mesenchymal transdifferentiation.

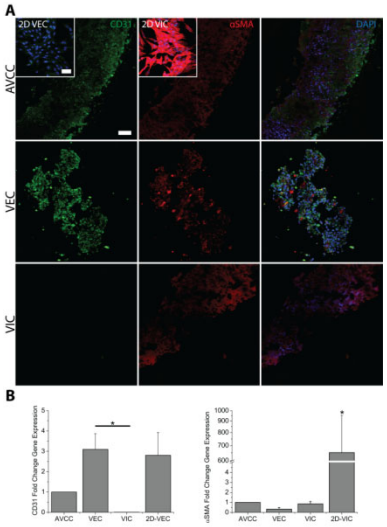

Figure 1. (A) IHC stains for αSMA (red) and CD31 (green) in the AVCC, 3D VIC and VEC cultures, and 2D VIC and VEC cultures (insets). Nuclei counterstained with DAPI (blue). Scale bar = 50 μm. (B) qRT-PCR results for the phenotypic markers αSMA and CD31 *: p<0.05 v. other groups. ^: p<0.05 within bracket.

Conclusions: This study is the first to assemble VICs and VECs into an engineered 3D model of the leaflet in hours without the need of a scaffold, while demonstrating physiological results like VIC quiescence and ECM formation. This model could be used as a basis for future approaches to tissue engineering, and demonstrates that scaffoldless approaches to heart valve tissue engineering are feasible and attractive. Furthermore, the AVCC could be used for a wide variety of experiments, such as those involving mechanobiology, or the progression of CAVD.