Valvular Interstitial Cell Response to Elasticity in Three-Dimensional Microenvironments

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Statement of Purpose: Valvular interstitial cells (VICs) are the primary cell type in the heart valve and play a major role in the progression of valve disease. In a healthy valve, the majority of VICs are quiescent fibroblasts, which can be activated to myofibroblasts. Myofibroblasts exhibit increased proliferation, cytokine secretion, and matrix remodeling. While this response is necessary for repair of valve tissue, pathological activation can lead to fibrosis and eventually calcification[1]. Researchers have examined this progression by using cell culture systems that allow for independent control of mechanical and biochemical factors. In two dimensions, substrate elasticity correlates strongly with VIC phenotype, where low substrate elasticity maintains the quiescent VIC phenotype and increasing substrate elasticity activates the VICs to the myofibroblast phenotype [2]. While there has been much progress in understanding how VICs respond to mechanical and biochemical cues in two dimensions, less is known about how these factors may influence phenotype in a three-dimensional environment. Changes in gene expression, cell morphology, polarity, cell-cell interactions, and cellular response to soluble factors have been observed in response to the dimensionality of the cellular microenvironment[3]. Here, we culture VICs encapsulated in hydrogels of varying moduli to explore the effects of microenvironment elasticity on VIC phenotype in three dimensions.

Methods: Passage 2 or 3 VICs were encapsulated in an 8-arm poly(ethylene glycol)-norbornene (PEG-norbornene) hydrogel crosslinked with a proteinase-degradable peptide (KCGPQGIWGQCK). The thiol-ene reaction was initiated with 365nm light (1.5 W/cm^2) and the photoinitiator lithium phenyl-2,4,6- trimethylbenzoyl-phosphinate (LAP). A pendant peptide, CRGDS, was incorporated to permit cell attachment. By changing the polymer molecular weight, amount of di-thiol crosslinker and initial polymer concentration, the moduli of the hydrogels was varied. For stiffening experiments, VICs were allowed to spread in soft hydrogels for 3 days. Then, cell-gel constructs were stiffened by diffusing in 8-arm PEG-norbornene, 8-arm PEG-thiol, and LAP and then exposing to light (365 nm, 1.5 W/cm^2). Controls were included to ensure that changes in phenotype were due to the increase in modulus and not exposure to radicals, ultraviolet light, or the stiffening solution. The moduli of the swollen hydrogels before and after stiffening were characterized using a rheometer equipped with an 8 mm parallel plate geometry. Cells were immunostained for α-smooth muscle actin (αSMA) and were characterized as myofibroblasts if αSMA stress fibers were present. Quantitative real-time polymerase chain reaction (qRT-PCR) was used to analyze the mRNA levels of αSMA.

Results: When encapsulated in hydrogels of a range of moduli, only VICs in the softest materials (Young's modulus, E = 0.2 kPa) formed αSMA stress fibers. Additionally, αSMA mRNA levels were inversely related to modulus. Cell morphology also differed in compliant gels compared to stiff gels (E > 3 kPa). VICs in the softest gels spread and elongated, while VICs in stiff gels remained smaller and rounded. To separate the effects of VIC morphology from the response to elasticity, VICs were encapsulated in soft gels, which were then stiffened to a medium (E = 1.2 kPa) or high (E = 13 kPa) modulus with high cell viability. VICs in stiffened hydrogels had similar morphology, with approximately equal aspect ratios and cell area. The inverse relationship between modulus and αSMA expression was maintained after controlling for morphology. VIC activation as determined by the presence of αSMA stress fibers also decreased with decreasing modulus.

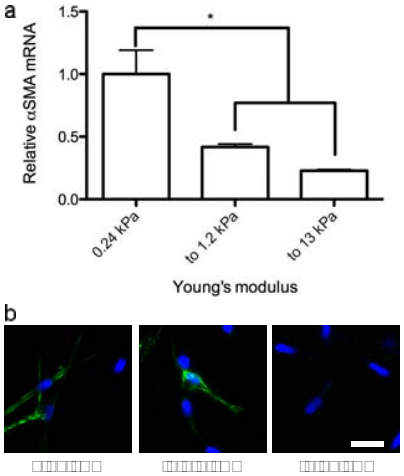

Figure 1. a) Levels of αSMA mRNA normalized to L30 housekeeping gene for encapsulated VICs that were allowed to spread in 0.24 kPa hydrogels before stiffening to their final modulus (n=2). b) Immunostaining for αSMA (green) and nuclei (blue) shows decreasing VIC activation with increasing modulus. Scale bar = 20 μm.

Conclusions: Results indicate VIC activation is inversely related to the modulus of the environment in 3D. This is in direct contrast to 2D studies showing that increasing substrate modulus increases activation. We have also found that this response to modulus is not a result of the inability of VICs to achieve an elongated morphology. We plan to investigate how VICs respond to biochemical cues, such as transforming growth factor beta, in 3D environments of varying moduli to assess whether elongated cells in stiff hydrogels can become activated.

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References: 1) Liu AC, Joag VR, Gotlieb AI. Am J Clin Pathol. 2007;171:1407-18. 2) Kloxin AM, Benton JA, Anseth KS. Biomaterials. 2010;31:1-8. 3) Baker BM & Chen CS. J Cell Sci. 2012;125:3015-24.