Hydrogels preserve native phenotypes of valvular fibroblasts through an elasticity-regulated PI3K/AKT pathway

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Statement of Purpose: Valvular interstitial cells (VICs) are the principal cellular component of cardiac valves and maintain normal valve homeostasis. During valvular fibrosis, VICs differentiate into myofibroblasts and stiffen the valve matrix. Myofibroblasts are characterized by increased contractility with α-smooth muscle actin (α-SMA)$^{+}$ stress fibers and increased secretion of extracellular matrix proteins, such as collagen type I. Myofibroblasts remodel damaged tissue and some later undergo apoptosis during normal wound healing. However, with dysregulation, myofibroblasts persist, proliferate and deposit excessive collagen, leading to tissue fibrosis. Stiffening of fibrotic tissue has been demonstrated to act as a biomechanical cue to promote myofibroblast survival and activation. Despite the recognized importance of matrix stiffness, or elasticity, in regulating the function and differentiation of fibroblasts and various other cell types, the effect of matrix stiffness is still debated and many questions remain about how the biomechanical cue of elasticity is translated into intracellular signaling. In this study, we utilize a photo-degradable poly(ethylene glycol) (PEG)-based hydrogel system to specifically explore how changes in substrate modulus regulate intracellular signaling, particularly the PI3K/AKT pathway.

Methods: Primary VICs were harvested from porcine aortic valves based on a sequential collagenase digestion. VICs were grown in Medium 199 with 15% fetal bovine serum in a standard cell incubator. PEG hydrogels were prepared as ~0.25 mm thin-films on coverglasses and made with two different moduli, ~7 kPa (soft) and ~32 kPa (stiff), or degraded *in situ* from ~32 kPa to ~7 kPa (stiff-to-soft) via 365nm UV light at 10mW/cm^2 for 5min (1). Cells cultured on different substrates were then fixed for immunocytochemistry analysis or lysed to collect RNA or protein for gene expression quantification based on RT-PCR or protein analysis based on western blot. Porcine genome microarrays (Affymetrix) were performed based on standard hybridization methods and analyzed using multiple bioinformatics tools (Genespring, DAVID functional annotation, Ingenuity pathway analysis). Experiments were based on at least 3 biological repeats and analyzed using a one-way ANOVA statistical test.

Results: In this study, we found that stiff TCPS (*E*, ~3 GPa) spontaneously activates the pathogenic myofibroblast phenotypes; whereas soft hydrogels (*E*, ~7 kPa) inhibit these traits (Fig. 1A and 1B). To understand how soft hydrogels preserve the un-activated fibroblast phenotype of VICs, we analyzed microarray data and found that the PI3K/AKT pathway is activated in VICs cultured on TCPS (Fig. 1C) and is necessary for myofibroblast activation on stiff substrates. Inhibition of PI3K with a small molecular inhibitor (LY294002) prevents myofibroblast differentiation and contraction-mediated nodule formation on both stiff gels and TCPS. Inhibition of AKT also inhibits some myofibroblast phenotypes. We also observed that, as myofibroblasts de-activate in response to reducing substrate modulus *in situ*, endogenous pAKT is decreased as early as 2 hours after gel softening and continues to decrease over a time course of 48 hours (Fig. 1D). Overexpression of constitutively active PI3K leads to increased αSMA protein expression and can drive myofibroblast-mediated nodule formation on soft hydrogels and TCPS.

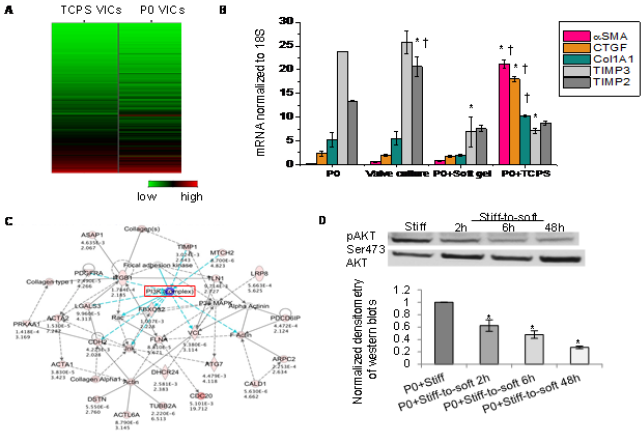

Figure 1. (A) Hierarchical clustering based on gene expression from the microarrays for P0 VICs and TCPS VICs. (B) VICs cultured on soft gels or in native valve matrix (valve culture) mimicked the gene expression of P0 VICs; whereas those cultured on TCPS showed up-regulated fibrogenic gene expression. * indicates significantly different from P0 with $p < 0.05$; † indicates significantly different from the soft gel condition with $p < 0.05$. (C) Ingenuity pathway analysis based on the differentially regulated genes from (A) revealed that the PI3K complex was up-regulation (red highlights). (D) pAKT/AKT, a downstream target of PI3K, was reduced 2, 6, and 48 h after softening the gels. *: $p<0.05$.

Conclusions: Our results suggest that VICs are sensing the changes in matrix elasticity through the PI3K/AKT pathway. This mechanism may be employed by other mechanosensitive cells in response to substrate modulus, and this pathway may be a worthwhile target for treating matrix stiffness-associated diseases, wherein the stiffened tissue reinforces myofibroblast activation. Furthermore, PEG hydrogels can be designed to recapitulate important mechanical cues in native tissues to preserve aspects of the native phenotype of primary cells for understanding basic cellular responses to biophysical and biochemical signals and for tissue engineering applications.

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References: 1. Kloxin AM, Tibbitt MW, Anseth KS. Nat. Protoc. 2010: 5(12): 1867-1887.