Isolating the Effect of Stiffness on Valvular Interstitial Cells and its Role in Phenotype Control

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Statement of Purpose: Examination of stiffness effects on valvular interstitial cells (VICs) has been measured in narrow range of moduli due to biomaterial fabrication limitations or have used different materials to model a larger moduli range without considering surface chemistry (1, 2, 3). For example when cultured on hydrogels with a limited tunable moduli (0.15-25 kPA) VICs become myofibroblastic shown by increased alpha smooth muscle actin (αSMA) expression (1, 2). As the moduli of specific layer of the aortic valve has been estimated to range from 29.55 to 137.5 kPA during compression and tension of the valve, respectively, a larger moduli range needs to be examined (4). Furthermore, changes in surface chemistry of the substrate and additional VIC disease markers were not examined. Therefore, a co-polymer system was developed in which moduli can be made to model early to late stages of disease aortic valve tissue, ranging from soft (25kPa) to hard (4.7 MPa) while presenting an unvaried surface chemistry.

Methods: Co-polymer was polymerized using monomers diethelyene glycol dimethacrylate (DEGDMA) and n-Octyl methacrylate (nOM) in 3:97, 19:81, and 33:77 ratios, with 0.0025% UV photcrosslinker Igacure 2959, crosslinked at 999 mj/cm^2 for 500 min. Samples were washed for 48 hr in ethanol, dried in vacuum, and immersed in milliQ H20. Compressive bulk modulus was measured following ASTM 695 – 02 standards. Surface chemistry was analyzed using x-ray photoelectron spectroscopy (XPS) and sessile drop goniometry. VICs were isolated using previously published collagenase digestion method and used at passage three (5). VICs were cultured on DEGDMA/nOM for a week. TaqMan primers on a StepOne qPCR system were used to measure gene expression at 3, 5, and 7 days (ΔΔCT method). Osteoblastic TCPS controls were grown in media supplemented with 10mM β-glycerophosphate, 10^{-6} M ascorbic acid, & 10^{-7} dexamethasone.

Results: DEGDMA/nOM formulations span a large range of moduli imitating different stages of aortic valve health and disease (Fig. 1A). Sessile drop goniometry shows substrates maintain the same contact angle and XPS values indicating surface composition remains consistent between formulations (data not shown). Quantitative PCR shows no difference in αSMA between all DEGDMA/nOM formulations at day 7 (Fig 2A). However expression of osteocalcin, a late stage marker of osteoblastic differentiation, is significantly increased on stiff 4.7GPA versus soft 25kPa substrates at day 7 (Fig. 2B). We also see an 8-16 fold increase in collagen expression on all DEGDMA/nOM substrates as compared to controls at day three (Fig. 2C). Furthermore, osteoblastic induction of VICs on TCPS using media express a ~4-5 fold increase in Elastin at day three as compared to all DEGDMA/nOM conditions and TCPS control (Fig. 2D).

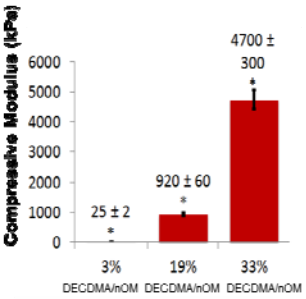

Figure 1. Moduli achieved using the 3:97 (3%), 19:81 (19%), and 33:77 (33%) DEGDMA/nOM formulations.

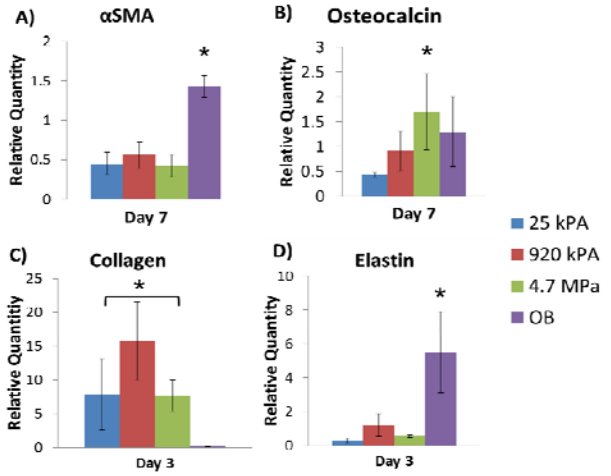

Figure 2. A) No difference in αSMA between DEGDMA/nOM substrates B) Increased OCN expression on 4.7 MPa DEGDMA/nOM at day 7 versus 25 kPa, C) increase collagen 1α expression on DEGDMA/nOM surfaces compared to controls and D) increase elastin expression of OB induced VICs at day 3.

Conclusions: Our copolymer system with unvaried surface chemistry and controlled modulus show stiffness ***alone*** can change VIC behavior and induce osteoblastic differentiation, indicated by expression of the osteoblastic marker osteocalcin, without increasing aSMA. VIC expression of collagen on the surface of DEGDMA/nOM is significantly higher (8-15 fold) compared to osteoblastic and normal TCPS controls. ECM components are known to influence VIC phenotype and therefore the choice of substrate to invoke the correct ECM protein production in experimental systems is crucial. Interestingly the elastin profile (Fig 2D) and αSMA (data not shown) of our stiffest substrate does not mimic the OB control at day three, suggesting stiffness may cause VIC OB differentiation through an alternative pathway to traditional OB media induction.

References: 1) Kloxin A. Biomaterials, 2010:31:1-8. 2) Quinlan A.M. Soc for Biomaterials 2012:100A:2474-2482. 3) Chen J. Circ Res. 2011;108:1510-1524 4) Buchanan R. Biomech Model Mechanobiol (2014) 13:813–826. 5) Johnson, C.M, J Mol Cell Card, 1987:19:1185-1193.