Substrate Surface Chemistry Directs Valvular Interstitial Cell Phenotypic Expression

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Statement of Purpose: Heart valve tissue engineering focuses on the use of valvular interstitial cells (VICs) due to their role in valve formation and tissue homeostasis. However, VICs exhibit a phenotypic plasticity with quiescent, activated, and osteoblastic (diseased) states.[1] The aim of this work is to examine the influence of physiologically relevant chemical functional groups on attachment, proliferation, and differentiation of VICs using self-assembled monolayers (SAMs) to create CH_3 (hydrophobic), OH (hydrophilic), COO^- (negative at physiological pH), and NH_2^+ (positive at physiological pH) surfaces. With changes in surface chemistry, it was hypothesized that different VIC phenotypes could be isolated for future study.

Methods: SAMs Fabrication & Characterization: SAMs were created and characterized as previously described.[2] **Cell Studies:** Primary VICs were obtained from porcine aortic heart valves via collagenase digestion.[3] Endothelial cells were removed using CD31-coated magnetic dnyabeads. Samples were seeded at 25,000 cells/cm^2 (n=5). Osteoblastic (OB) cells were cultured in 10mM β-glycerophosphate, 10^{-6} M ascorbic acid, & 10^{-7} dexamethasone.[4] Proliferation was determined using MTT cell proliferation assay (ATCC, 30-1010K) after 12 hours (attachment) and 3, 5, and 7 days. Immunocytochemical staining of alpha-smooth muscle actin (αSMA, ab7817), collagen-I (ab90395), and elastin (ab21610) was conducted to examine cell attachment, spreading, and tissue deposition. Genetic analysis (qPCR) of αSMA (activation), prolyl-4-hydroxylase (P4H, tissue deposition), and bone gamma-carboxyglutamic acid-containing protein (BGLAP/osteocalcin, disease) was conducted using TaqMan probes with glyceraldehydes 3-phophatase dehyderogenase (GAPDH) as the endogenous control. Calcium deposits were stained with 10% alizarin red.

Results: Characterization: XPS characterization and contact angle measurements (107°±1, 23°±1, 43°±3, & 26°±2 for CH_3, OH, NH_2^+, & COO^- surfaces, respectively) confirm SAM formation and correspond with published values.[2]

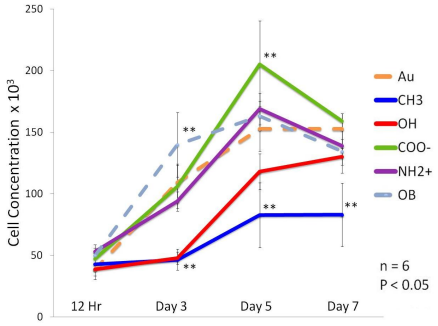

Figure 1. Proliferation on SAMs compared to Au and osteoblastic (OB) controls. Rapid initial OB proliferation is soon overtaken by COO^- &NH_2^+ SAMs while proliferation is reduced on OH (recovered) & CH_3 SAMs.

Cellular Studies: Attachment and proliferation of VICs occurred on all surfaces to varying degrees (Fig. 1). Early proliferation was exhibited by cells in OB media as expected[5], followed by increases on COO^- and NH_2^+. VICs exhibited normal spreading and morphology on all surfaces at day 5 except NH_2^+, on which they exhibited a cuboidal shape similar to osteoblastic VICs (Fig. 2a-b). Increases in osteocalcin expression (Fig. 2d), collagen-I deposition (Fig. 2c), and the appearance of calcified nodules (Fig. 2b, inset) on NH_2^+ surfaces imply a rapid transition to an osteoblastic phenotype. While CH_3 environments also cause calcification, the lack of genetic markers for VIC osteoblastic differentiation implies an apoptotic mechanism of calcification. While cells on OH and COO^- surfaces (data not shown) are well spread and express high quantities of αSMA, a common indicator of activation, minimal tissue deposition suggests possible secondary signaling required for tissue formation.

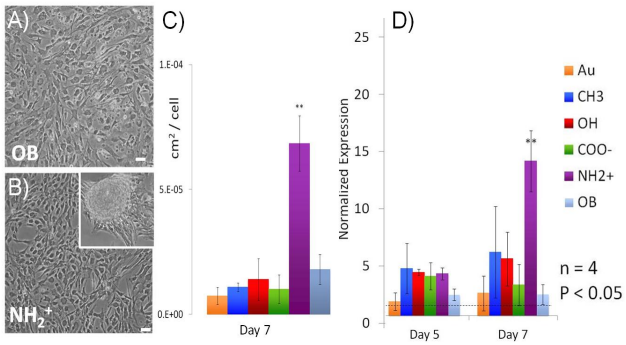

Figure 2. VICs grown A) in OB media and B) on NH_2^+ SAMs show early calcified nodule formation (inset) and similar cuboidal morphology, as compared to normal VIC elongated spindle morphology. C) Collagen-I deposition and D) BGLAP/osteocalcin genetic expression. Scale=50 µm

Conclusions: Cells on NH_2^+ surfaces exhibit differentiation towards an osteoblastic phenotype, expressing morphological indicators of disease and early expression of osteocalcin as compared to VICs in osteogenic media conditions. These results suggests that NH_2^+ surfaces induce differentiation at a faster rate without the use of exogenous signaling factors, and could provide an alternative method to study mechanisms of disease formation due to surrounding cellular environment. Conversely detachment and aggregation of cells from hydrophobic CH_3 surfaces indicates clustered nodule formation and calcification through an apoptotic mechanism.[4] OH and COO^- surfaces show minimal tissue deposition thus far, however a focus on secondary signaling should be further examined.

References: 1) Liu AC, Am J Path. 2007, 171:1407-1416. 2) Keselowsky BG, JBMR, 2003, 66A:247-259. 3) Johnson CM, J Mol Cell Card, 1987, 19:1185-1193. 4) Cloyd KL, PLOSone, 2012, 7:e48154. 5) Monzack EL, J Heart Valve Dis., 2011, 20:449-463.