Surface Chemistry Modulation of Valvular Interstitial Cells

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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 tissue formation and homeostasis. However, VICs can exhibit a phenotypic plasticity of quiescent, activated, and osteoblastic (diseased) states.[1] The aim of this work is to examine the influence of physiologically relevant functional groups for their control over 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. These results will influence the intelligent design of scaffold materials for tissue regeneration by creating the most favorable growth environment.

Methods: **SAMs Fabrication:** Microscope cover glass was etched in Piranha for 30 minutes followed by evaporation of chromium (15 Å) and gold (300Å). Freshly prepared coverslips were immersed in 1mM alkanethiols (1-dodecanthiol; 11-mercapto-1-undecanol; 11-mercaptoundecanioc acid; 11-amino-1-undecanethiol, hydrochloride 1N NaOH) in absolute ethanol overnight.[2]
Surface Characterization: To determine surface functionalization, x-ray photoelectron spectroscopy (XPS) was performed. Sessile drop goniometry was used to measure contact angle. Charge functionality of COO^- and NH_2^+ SAMs was determined with Raman spectroscopy.
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). Proliferation was determined using MTT cell proliferation assay (ATCC, 30-1010K) after 12 hours (attachment) and 3, 5, and 7 days. Immunocytochemical staining of vinculin (Abcam, ab73412) and alpha-smooth muscle actin (a-SMA, Abcam, ab7817) was conducted to examine cell attachment, spreading, and phenotype.

Results: **Characterization:** XPS scans indicate good formation of SAMs compared to theoretical (Tables 1), and reported results.[2] Contact angle measurements (107°±1, 23°±1, 43°±3, & 26°±2 for CH_3, OH, NH_2^+, & COO^- surfaces, respectively) confirm XPS results and correspond with published values.[2]

Table 1. XPS measurement of surface chemistry composition on SAMs.

	Composition (at. %)							
	Theoretical				Actual (30° toa)			
	C_{1s}	O_{1s}	N_{1s}	S_{2p}	C_{1s}	O_{1s}	N_{1s}	S_{2p}
CH_3	92.3			7.7	95.8	0.9		3.3
OH	84.6	7.7		7.7	85.9	10.7		3.4
COO^-	80	13.33		6.67	85.9	10.2		3.9
NH_2^+	84.6		7.7	7.7	84.9	6.4	5.8	2.9

*toa: take-off angle

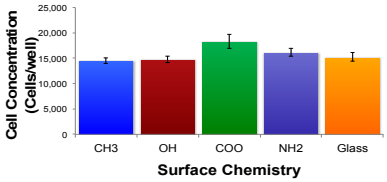

Figure 1. Cell attachment on functionalized surfaces after 12 hours.

Cellular Studies: Results show, surface chemistry does not significantly influence VIC attachment to SAMs. Cellular attachment and spreading on COO^- functionalized surfaces indicates a favorable environment for cell activation and future tissue deposition. Conversely, the aggregation of cells on CH_3 surfaces indicates the onset of osteoblastic nodule formation. Staining of activated or diseased phenotypes through a-SMA expression (Fig. 2, green) and vinculin (Fig. 2, red) surface binding further corroborate these results qualitatively showing an increase in expression on COO^- surfaces and reduction on CH_3.

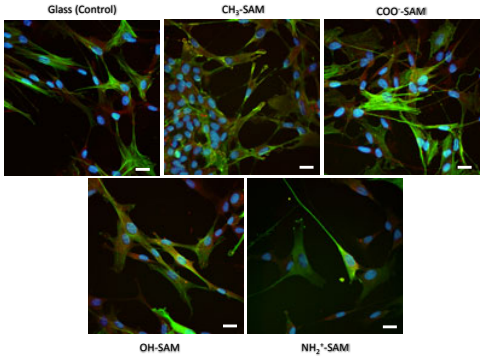

Figure 2. Immunocytochemical staining of VICs for vinculin (red) and a-SMA (green) after 24 hours of culture on SAMs. Scale = 20 µm

Conclusions: Based on above results, all surfaces support VIC attachment. However, COO^- surfaces exhibit greater spreading, indicating differentiation towards the activated phenotype necessary for tissue regeneration, while avoiding morphological indicators of disease. Conversely aggregation on hydrophobic CH_3 surfaces suggests the onset of disease progression normally seen as clustered nodule formation that may lead to later stages of tissue calcification. Future experiments will focus on quantitatively determining phenotype using real time polymerase chain reaction (RT-PCR) to measure expression of a-SMA (activation), prolyl-4-hydroxylase (ECM deposition), and osteocalcin (osteoblastic disease state), to identify activated tissue depositing states while minimizing calcification.

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.