Appendix

Surface Chemistry Regulates Valvular Interstitial Cell Differentiation In Vitro

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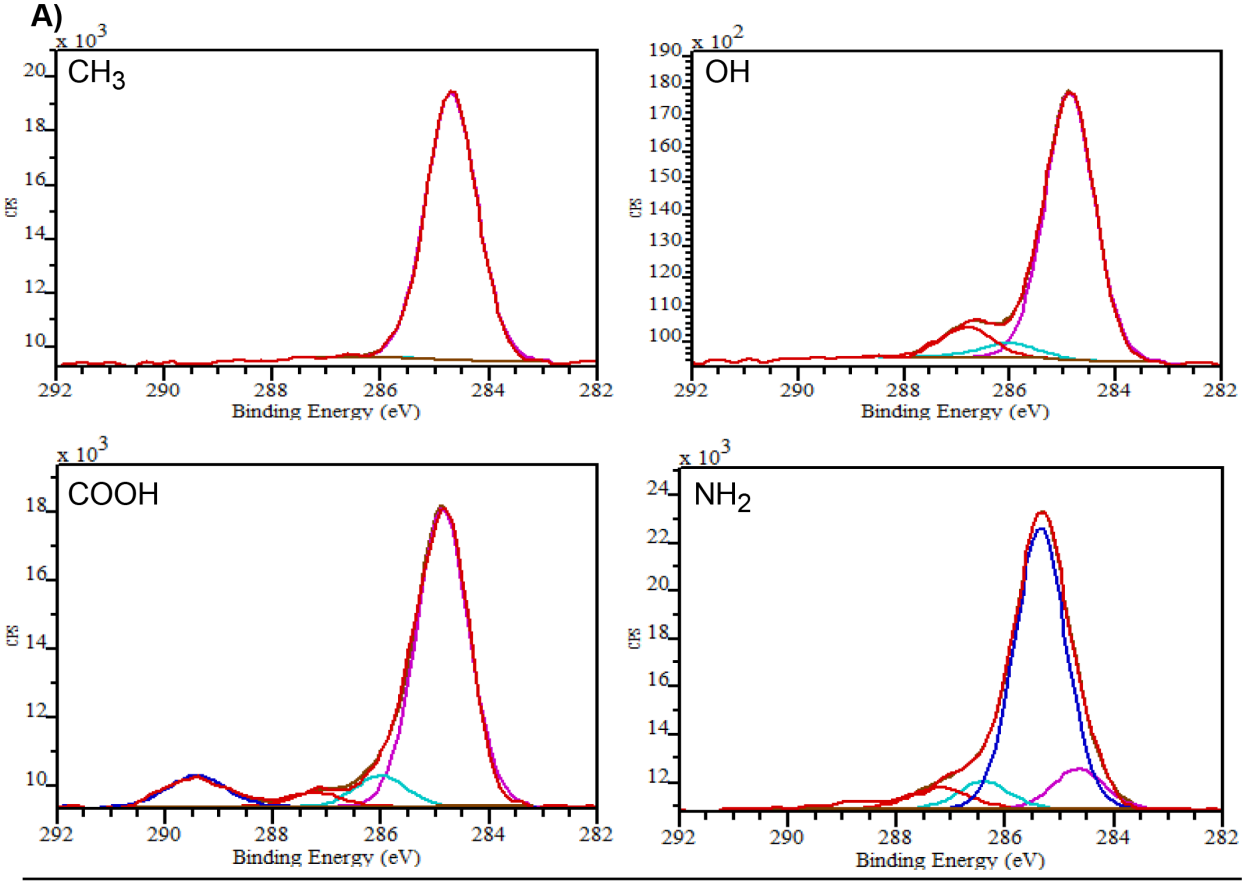
Supplemental Figures

A)



B)

	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
COOH	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 I. A) XPS detail spectra and B) chemical composition for ω-functionalized alkanethiol self-assembled monolayers. The lack of oxygen on CH3-SAMs, increased C-OH peak at 287.0 eV on OH-SAMs, further increases in O (COOH at 289.4 eV) on COOH-SAMs, and presence of nitrogen (C-N at 285.4 eV) on NH2-SAMs all agree with monolayer formation, as well as theoretical values.

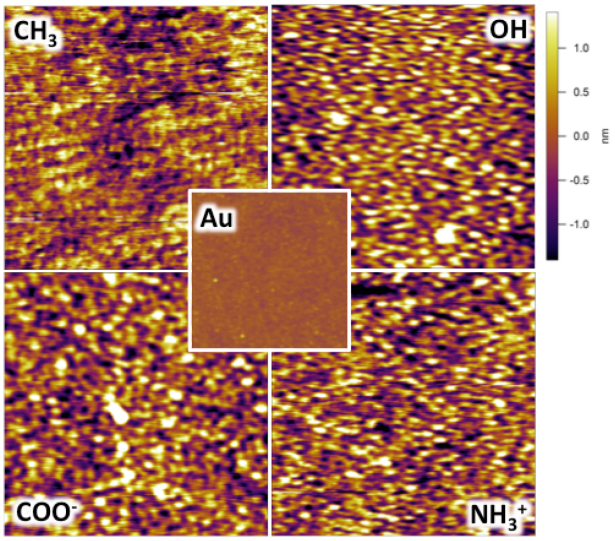


Figure II. Surface topography obtained through atomic force microscopy (AFM) of functionalized SAMs compared to Au controls. Images show monolayer formation with minimal variation in height. 20 μm Scan size.

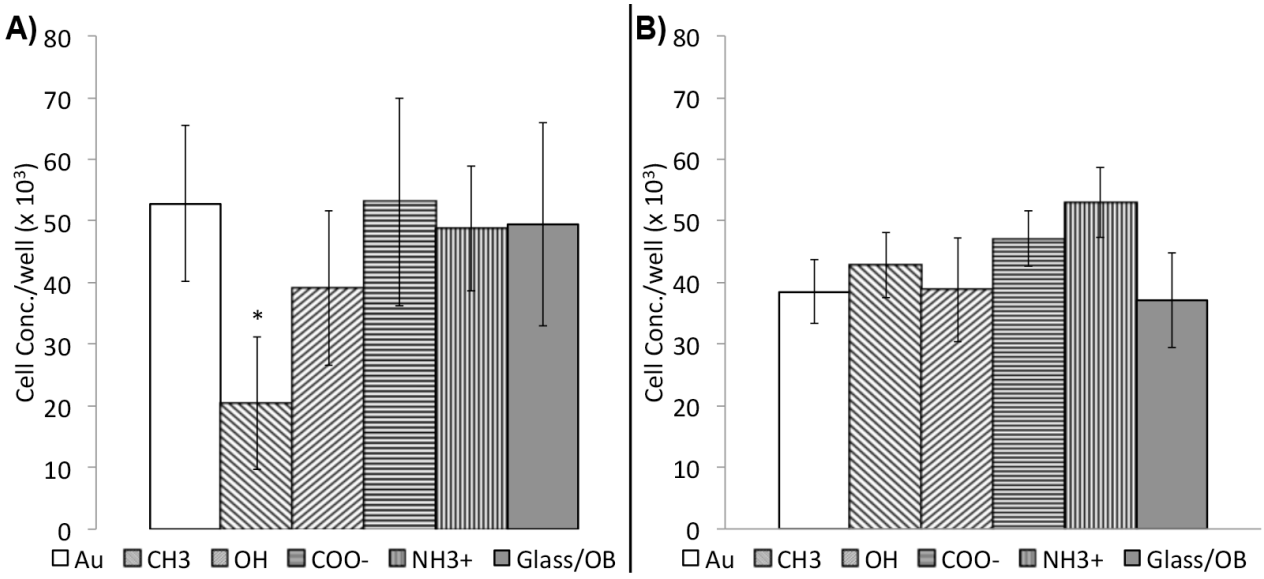


Figure III. Valvular interstitial cell (VIC) density upon attachment (12 hrs) on varying surface functionalities, as compared to bare glass/osteoblastic (OB) and gold controls. A) Statistically significant lower VIC attachment observed on CH3 SAMs when seeded (t=12 hr) at 21,500 cells/cm2 as compared to other functional groups and controls. B) Uniform seeding density achieved when seeded with 43,000 cells/cm2 on CH3-SAMs and 21,500 cells/cm2 on all other surfaces.

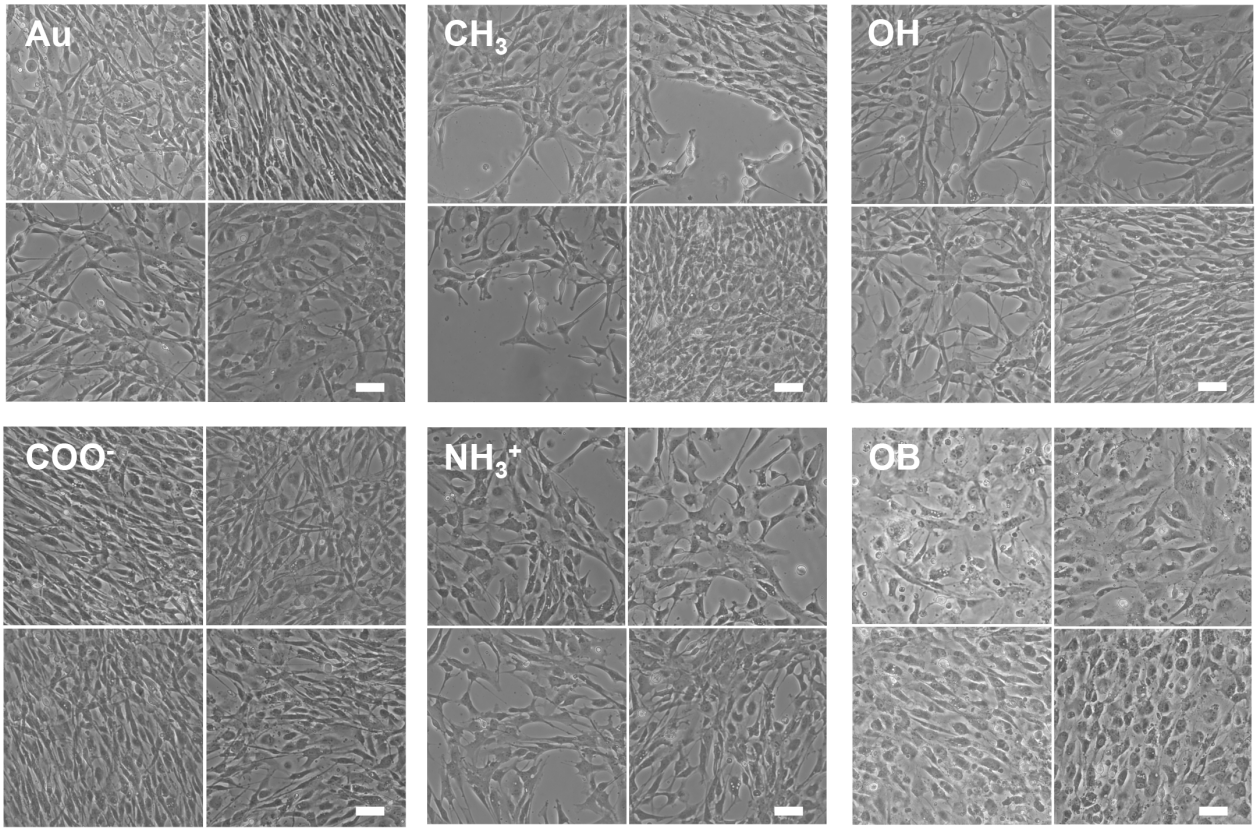


Figure IV. Representative images of VIC morphology on SAMs and control surfaces after 5 days in culture. Variations in individual morphology amongst VICs on SAMs are represented within populations. Scale = 50 μm

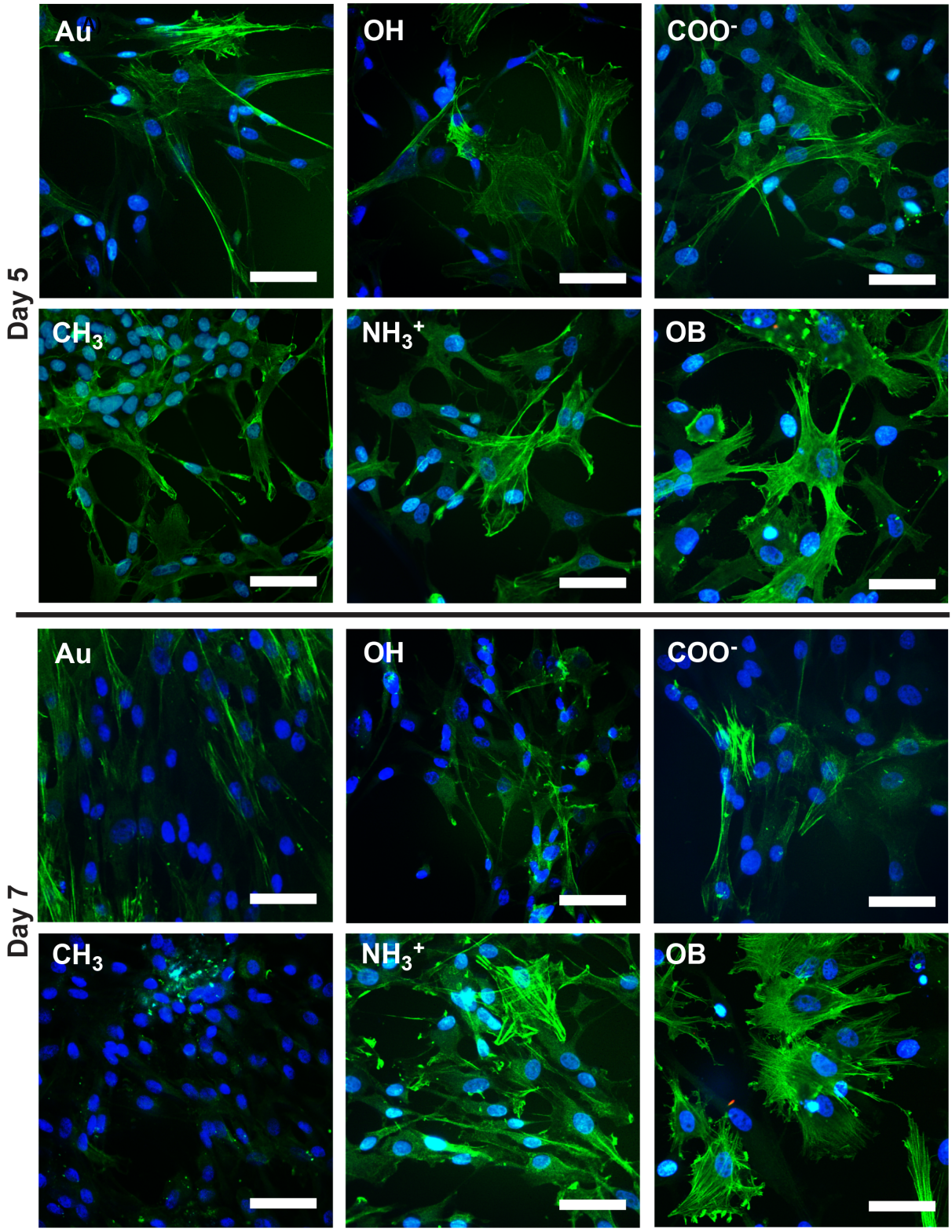


Figure V. Representative images of immunofluorescent stained α-smooth muscle actin cytoskeletal protein (green) with Dapi stained nuclei (blue) in VICs after five (top) and seven (bottom) days. Scale = 50 μm.

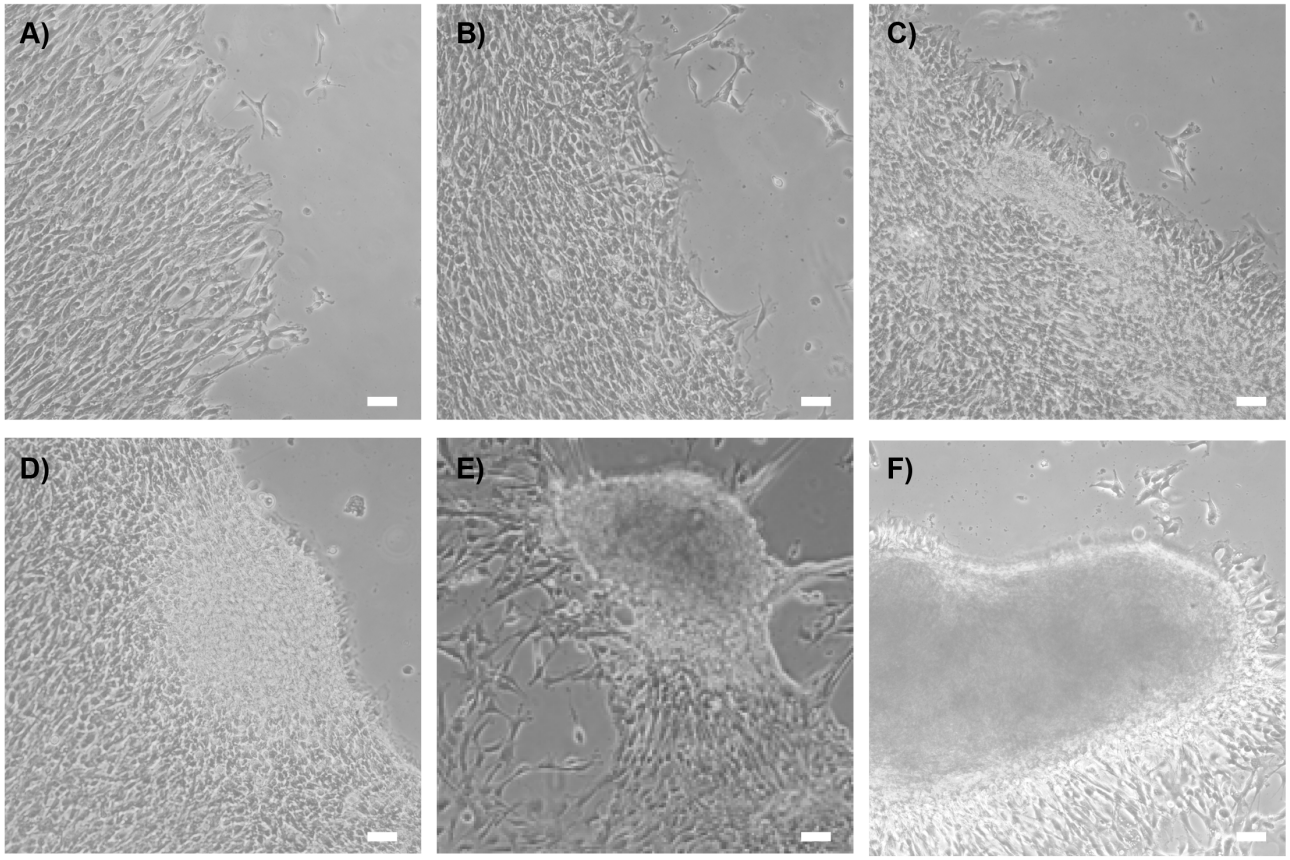


Figure VI. Representative images of cellular aggregation and detachment (cell sheet formation) on CH3-SAMs after 7 days in culture. Images show several stages of aggregates formed, beginning with detachment of VICs on edges of sheet (A-C), during media changes, and eventual folding/condensing of cell sheet onto remaining cells in culture (D-F). Scale = 50 μm

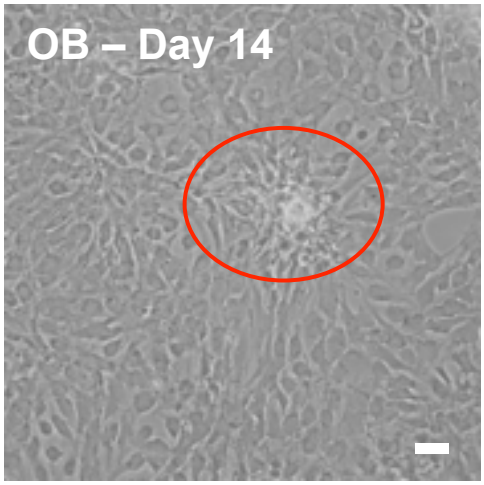


Figure VII. Representative images of cellular aggregation/nodules in osteoblastic culture after 14 days of growth. Scale = 50 μm

Supplemental Tables

Table 1. Air-water-surface Contact Angle Measurements and Thickness of CH_3, OH, COO^-, and NH_3^+-SAMs

	CH_3	OH	COO^-	NH_3^+
Contact Angle (˚)	105.9 ± 0.9	14.9 ± 1.5	31.8 ± 2.9	46.6 ± 4.9
Thickness (nm)	1.66 ± 0.02	1.43 ± 0.05	2.21 ± 0.05	1.61 ± 0.36