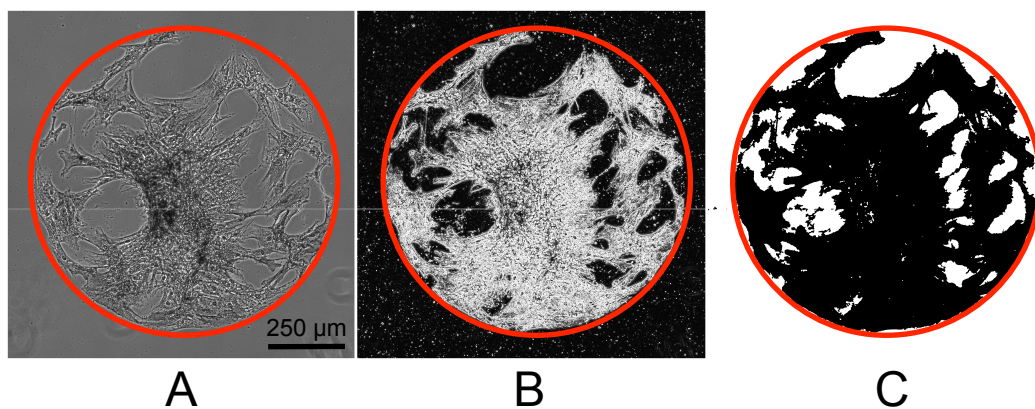
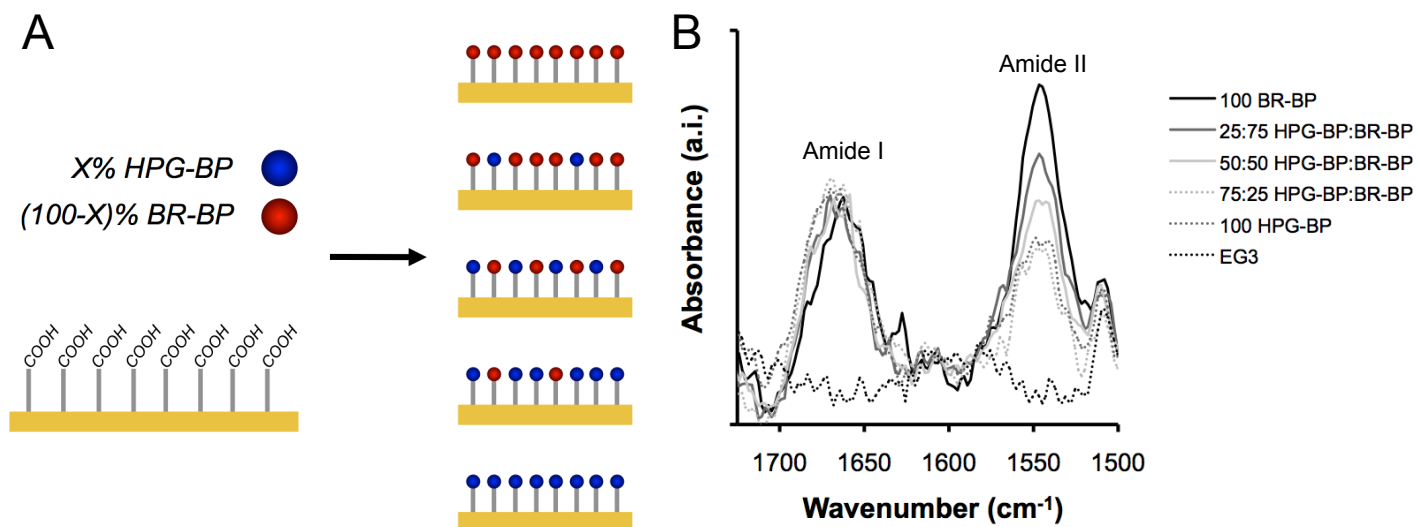


Supplement Figure 1. Generating defined culture substrates using alkanethiolate self-assembled monolayer arrays. (A) SAM arrays were generated using a simple elastomeric stencil adhered to the a gold substrate. (B) Schematic representation of SAM array fabrication: (1) Adhere elastomeric stencil to gold substrate to generate a microwell array superstructure, (2) locally form a SAM in each well with alkanethiolate mixtures containing carboxylic acid-terminated and hydroxyl-terminated oligo(ethylene-glycol) alkanethiolates, (3) covalently conjugate peptides to array spots via carbodiimide condensation of peptide n-terminal primary amine and SAM carboxylic acid terminal moieties, and (4) remove mask and backfill with inert SAM.



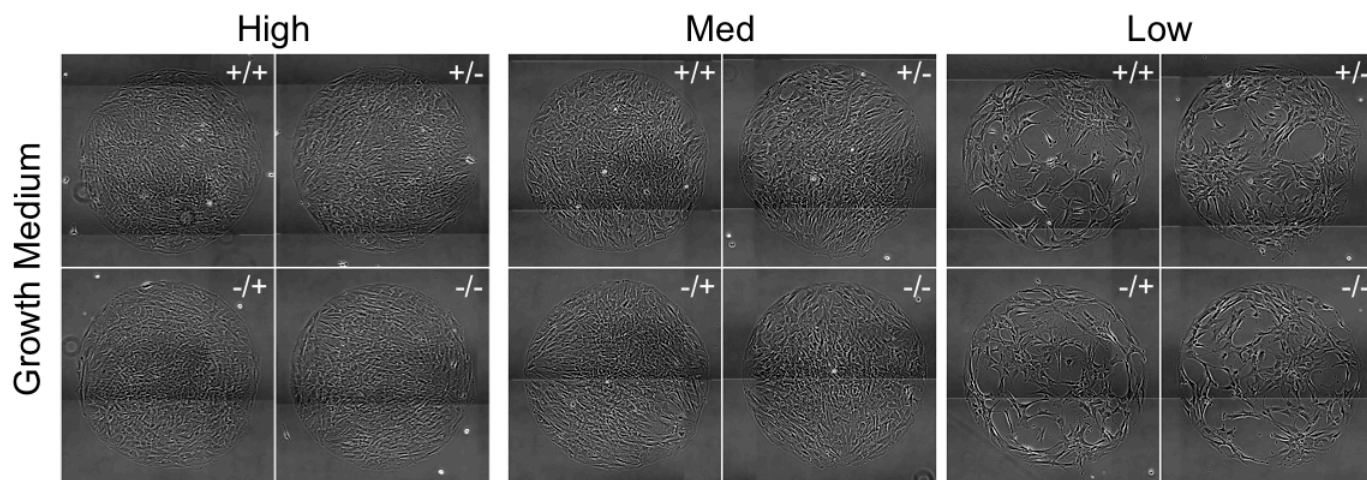
Supplemental Figure 2. Quantification of hMSC surface coverage on SAM arrays using image processing. (A) Example image of array spot with attached hMSCs. Nikon NIS Elements Software (Melville, NY) was used to perform (B) edge detection and (C) subsequent thresholding on images of array spots to calculate hMSC surface coverage within the region of interest (ROI) indicated as a red circle.



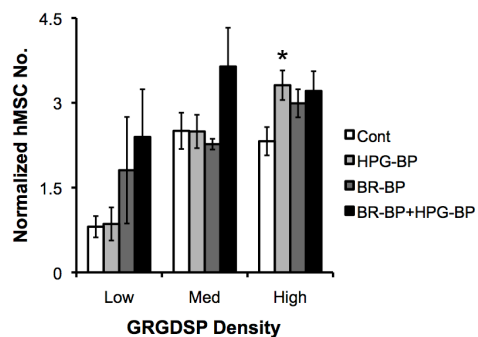
Supplemental Figure 3. Infrared analysis of mixed peptide immobilization. (A) Schematic of mixtures of the heparin proteoglycan-binding peptide (HPG-BP, 1165 Da) and the BMP-2 receptor binding peptide (BR-BP, 2290 Da) conjugated to carboxylate terminated SAMs. (B) PM-IRRAS spectra of HPG-BP and BR-BP mixtures conjugated to carboxylic acid terminated SAMs as well as a reference spectra of a 100% HS-C₁₁-EG₃-OH (EG3) SAM.

Polarization modulation-infrared reflection-absorption spectroscopy

Polarization modulation-infrared reflection-absorption spectroscopy (PM-IRRAS) was used to confirm peptide conjugation, via carbodiimide chemistry, to carboxylate-terminated SAMs. Additionally, PM-IRRAS was used to evaluate immobilized peptide composition. To achieve this, bulk gold chips (1000 Å Au <111>, 50 Å Ti on 1" x 3" X 0.040" glass slides were purchased from Evaporated Metal Films, Inc., Ithaca, NY. Cat. No. TA134) were prepared following the same steps described in the array fabrication process, but without use of the elastomeric stencil. IR spectra of alkanethiolate SAMs on 1000 Å Au films were obtained using a Nicolet Manga-IR 860 FT-IR spectrometer with a photoelastic modulator (PEM-90, Hinds Instruments, Hillsboro, OR), synchronous sampling demodulator (SSD-100, GWC Technologies, Madison, WI), and a liquid-N₂-cooled mercury telluride (MCT) detector. All spectra were obtained at an incident angle of 83° with modulation centered at 1500 cm⁻¹ and 2500 cm⁻¹ to produce spectrum with a range of 1000-3000 cm⁻¹. For each sample, 1000 scans were taken using a resolution of 4 cm⁻¹ per modulation center. Data were acquired as differential reflectance (%ΔR/R) versus wavenumber, and baseline correction was performed as outlined by Skoda, M.W.A., et al., *Optimizing the PMIRRAS signal from a multilayer system and application to self-assembled monolayers in contact with liquids*. Journal of Electron Spectroscopy and Related Phenomena, 2009. **172**(1): p. 21-26.



Supplemental Figure 4. hMSC surface coverage on SAM arrays presenting mixtures of BR-BP and HPG-BP and varied densities of GRGDSP (low, medium, high) at day 1. 1×10^6 hMSCs were seeded onto SAM arrays. (“-/-” Control, “+/-” BR-BP, “-/+” HPG-BP, “+/+” BR-BP and HPG-BP).



Supplemental Figure 5. hMSC proliferation on SAM arrays presenting mixtures of BR-BP and HPG-BP and varied densities of GRGDSP in growth media. 50,000 hMSCs were seeded onto SAM arrays and media was changed every other day. Normalized cell number was determined by dividing the cell number after 5 days by the cell number at day 1 (Error bars represent standard error of the mean and asterisk indicates significance difference compared to control within a GRGDSP density, $p < 0.05$, $n = 9$).