Patterning discrete stem cell culture environments via

localized SAM replacement

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Supporting Information

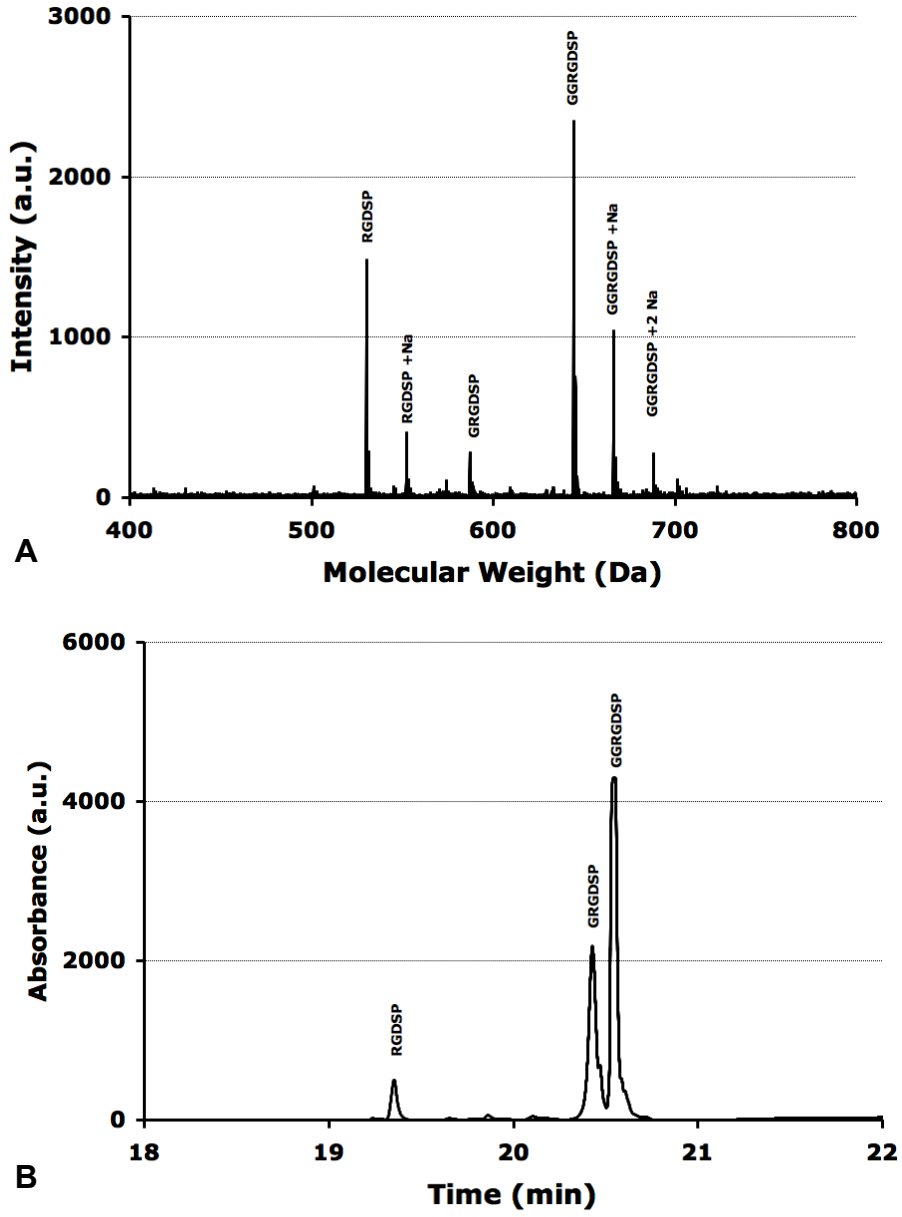

Figure 1. Characterization of synthesized RGDSP peptide. (A) MALDI-MS spectra of RGDSP peptide. (B) HPLC analysis of RGDSP peptide.

HPLC spectra indicated that the synthesized peptide stock was composed of ~5.5% RGDSP, ~35.5%GRGDSP, and ~59% GGRGDSP. Since all peptides included the full RGDSP sequence, the stock was used without further purification. Furthermore, this peptide stock was used to prepare all cell culture surfaces via conjugation to HS—EG_6—COOH molecules in a HS—EG_3—OH. The EG_6 unit provided an additional EG_3 spacer beyond the surrounding HS—EG_3—OH background.

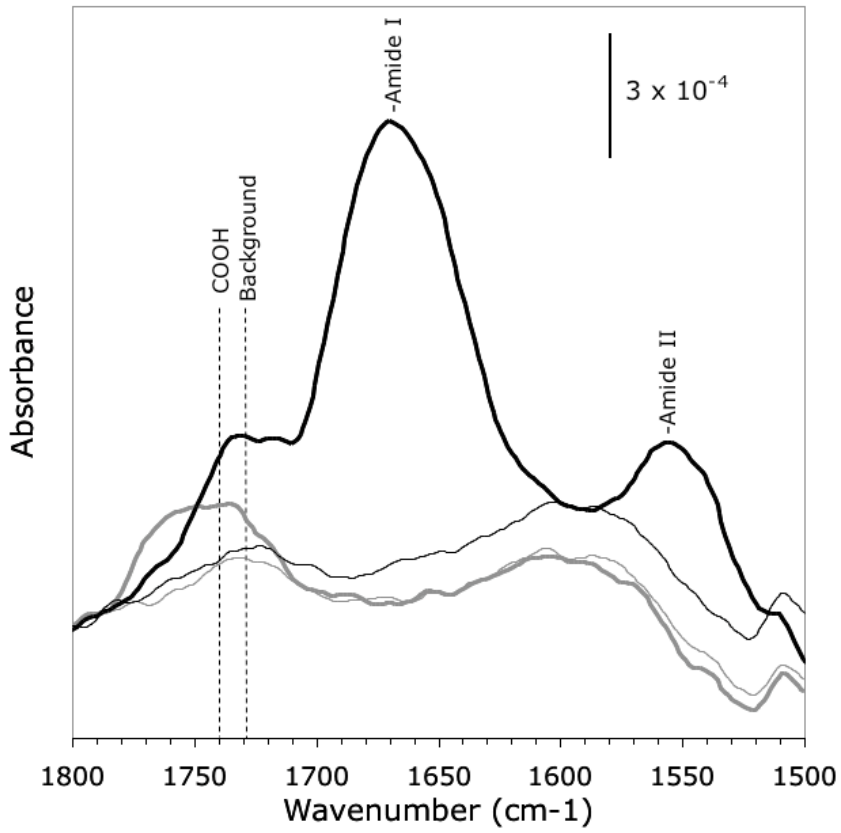

Figure 2. Stacked view of the carboxylic acid (1740 cm^{-1}), amide I (1675 cm^{-1}), and amide II (1550 cm^{-1}) stretch band regions of bare Au (——), 100% HS—EG_3—OH SAM (——), following SAM re-formation with 25 mol percent HS—EG_6—COOH solution (——), and following RGDSP peptide conjugation (——) spectra.

Due to a background peak centered around ~1730 cm^{-1} observed in both bare gold and 100% HS—EG_3—OH, 25 mol percent HS—EG_6—COOH conditions were chosen to enable clear observation of the carboxylic acid stretch band centered around 1740 cm^{-1}. The presence of this background peak made it difficult to assess whether peptide conjugation proceeded to completion. In particular, the amide I side peak observed between ~1730 and 1740 cm^{-1} in the spectrum following conjugation of RGDSP could be due to the background peak, or due to residual un-reacted carboxylic acid moieties. A theoretical calculation assuming an alkanethiolate spacing of 4.97 Å (Strong, L.; Whitesides, G., Structures of self-assembled monolayer films of organosulfur compounds adsorbed on gold single crystals: electron diffraction studies. *Langmuir* 1988, 4, (3), 546-558.), and estimating each peptide molecule to be a hard sphere with an approximate volume of 750 $Å^3$ would predict peptide surface saturation at ~20% with ~5% of un-reacted carboxylic acid.

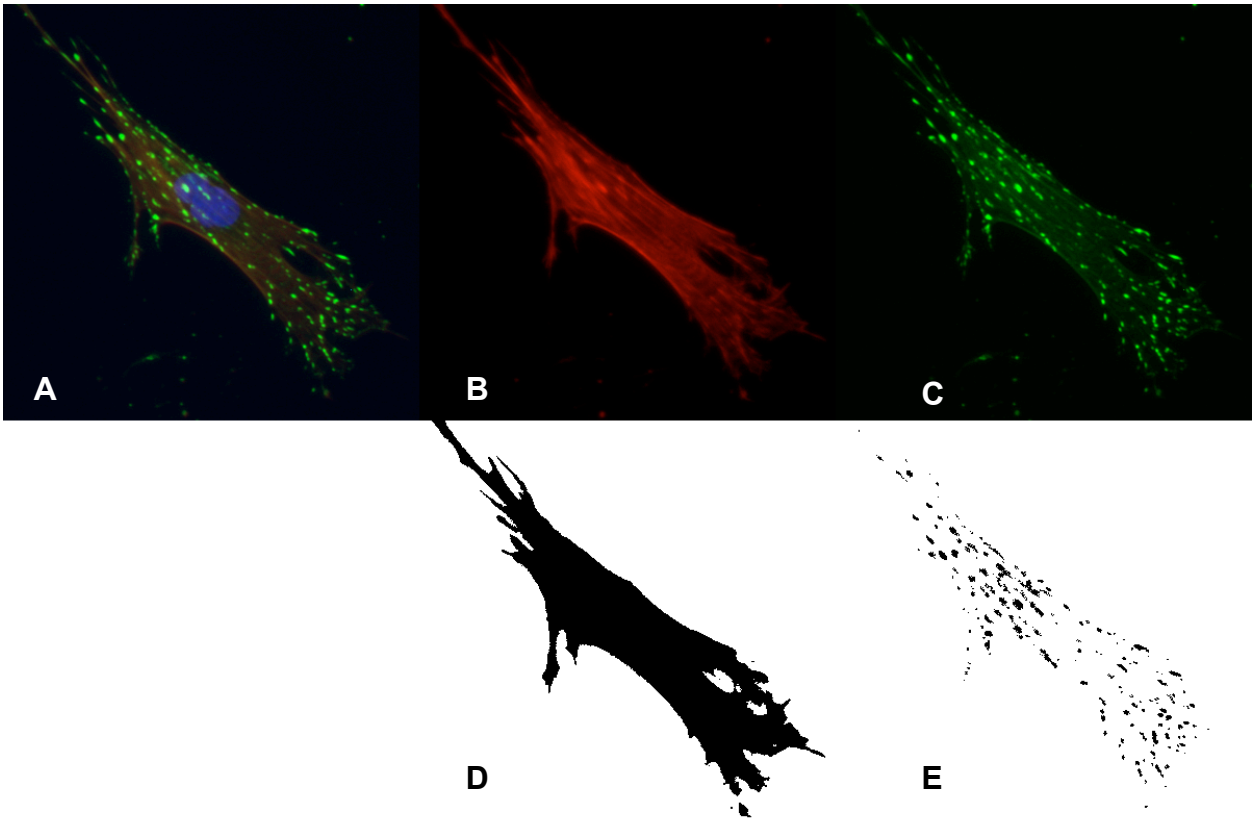

Figure 3. Example of image processing method used to quantify focal adhesion density. (A) Original image (B) Red channel – actin staining (C) Green channel –vinculin staining (D) Thresholded red channel (E) Thresholded green channel.