Chemoselective immobilization of multiple distinct peptides on SAMs for stem cell culture

Gregory A. Hudalla[a], William L. Murphy[a,b]

Department of Biomedical Engineering[a], Department of Pharmacology[b], University of Wisconsin-Madison, Madison, WI

Introduction: Self-assembled monolayers (SAMs) of alkanethiolates on gold are useful substrates to characterize cell-biomaterial interactions. For example, significant recent work has relied on SAMs presenting cell adhesion epitopes in an otherwise bio-inert background to characterize cell attachment, spreading, and focal adhesion complex formation. In many of these experiments, a biomolecule (e.g. peptide or protein) is covalently immobilized onto a SAM by reacting a moiety present in the biomolecule (e.g. NH_2) with a moiety present on the SAM (e.g. COOH). To date, this methodology has allowed for detailed characterization of the influence of a single biomolecule on cell behavior. However, the native extracellular environment is a complex network displaying multiple distinct cues that act in concert or antagonism to direct cell behavior. We have developed substrates that allow for covalent immobilization of two distinct peptides onto a single SAM substrate. Our approach relies on SAMs presenting orthogonally reactive carboxylate and azide moieties that allow for conjugation of amine- and acetylene-terminated peptides, respectively. Here we report the use of this strategy to immobilize the cell adhesion epitope, Arg-Gly-Asp-Ser-Pro (RGDSP), and a non-functional peptide, Arg-Gly-Glu-Ser-Pro (RGESP). Moreover, we demonstrate that mesenchymal stem cell (MSC) spreading and focal adhesion complex formation are dependent on RGDSP surface density, but are independent of RGESP density. We envision that this approach will be amenable to any biomolecules bearing an amine or acetylene moiety, and will allow for detailed characterization of the role of multiple distinct extracellular signals on cell function.

Methods: The peptides hexynoic acid-RGDSP and RGESP were synthesized using a standard Fmoc solid-phase peptide synthesis protocol. Tri(ethylene glycol)undecanethiol was synthesized using a standard protocol. Carboxylate-terminated hexa(ethylene glycol)undecanethiol was from Prochimia (Sopot, Poland). SAMs were formed by incubating gold-coated substrates overnight in an ethanolic solution containing alkanethiols. RGESP was immobilized onto SAMs by first incubating SAMs in an aqueous solution of N-hydroxysuccinimide and 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide, followed by incubation of SAMs in a 1x phosphate-buffered saline solution (pH 7.4) containing peptide. Hexynoic-acid RGDSP was immobilized onto SAMs by incubating SAMs in a solution of DMSO:HEPES (pH 8.5) (50/50, v/v) containing hexynoic acid-RGDSP, Cu(I)Br, sodium ascorbate, and Tris[(1-benzyl-1H-1,2,3-triazol-4-yl)methyl] amine.

Results: Polarization-modulated infrared reflectance absorbance spectroscopy (PM-IRRAS) was used to characterize SAMs presenting orthogonally reactive carboxylate and azide moieties (Fig. 1A-B), immediately after SAM formation, after conjugation of amine-terminated RGESP to surface carboxylate groups, and after conjugation of acetylene-terminated RGDSP to surface azide groups. We have recently demonstrated that MSC spreading and focal adhesion complex formation are dependent on RGDSP surface density[1]. In the current study we again characterized the correlation between RGDSP surface density and MSC behavior, however, the substrates also presented the non-functional peptide RGESP at co-varying molar ratios to elucidate the influence of a second peptide on RGDSP bio-activity. Our results (Fig. 1C) demonstrated that MSC spreading is dependent on RGDSP surface density, but independent of RGESP surface density. Additionally, our results (Fig. 1D) also demonstrated that focal adhesion complex density is dependent on RGDSP surface density, similar to SAMs presenting RGDSP alone.

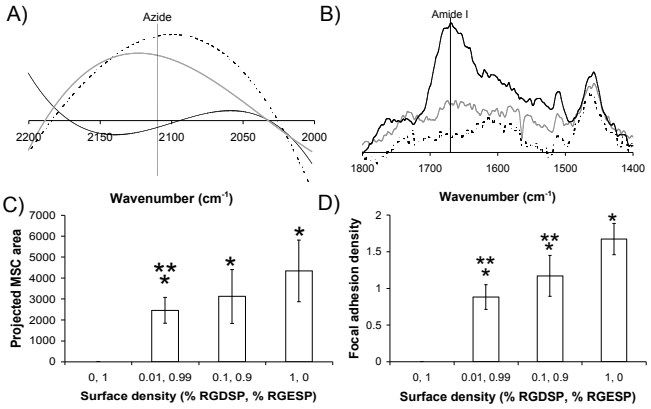

Figure 1: PM-IRRAS spectra over the range of (A) the azide functionality and (B) the amide I functionality after SAM formation (·····), after RGESP conjugation to surface carboxylate groups (—), and after immobilization of RGDSP to surface azide groups (—). Correlation between RGDSP surface density and (C) MSC spreading or (D) focal adhesion complex density. * represents significant difference compared to 0% RGDSP, ** represents significant difference compared to 1% RGDSP ($p < 0.05$)

Conclusion: We have demonstrated chemoselective immobilization of two functionally distinct peptides onto SAMs presenting orthogonally reactive moieties. Additionally, our results demonstrate that the presence of a second, non-functional peptide does not influence the bio-activity of the cell adhesion epitope RGDSP. Moreover, our results indicate that the presence of a second peptide does not influence the bio-inert nature of the underlying SAM substrate. The observed maintenance of RGDSP bio-activity in the presence of a second non-functional peptide identifies SAMs presenting orthogonally reactive moieties as promising base materials to characterize the influence of multiple distinct biochemical signals on MSC behavior.

References: 1) Hudalla, G.A.; Murphy, W.L. *Langmuir* 25:5737-46 **2009**.