594h Quantitative Adhesion Requirements for Intracellular Signaling, Cell Spreading and Cell Proliferation

Niki Galownia, Melissa Davis, David Tirrell, and Anand R. Asthagiri

Natural matrix proteins display a rich content of biochemical information, typically arranged in an elegant modular format. The tripeptide epitope (RGD) has garnered the most attention as a modular unit that promotes cell adhesion in a wide range of cell types. However, natural ECM proteins contain numerous non-RGD modular units. Principles for designing composite surfaces that mix RGD and non-RGD units to most effectively control cell adhesion, signaling and higher-order behaviors remain to be established. Our lab seeks to elucidate quantitatively the signaling and functional potential of non-RGD epitopes. Importantly, while ECM protein structure may be modular, the intracellular signaling and cell behavioral consequences of adhesion modules are often synergistic, as we show in this work focused on the contributions of RGD and the heparin-binding domain (HBD) on cell adhesion and spreading.

Using cell lines with distinct adhesion receptor (a5b1 integrin) expression profiles, we demonstrate that this integrin mediates cell spreading on substrata coated with genetically-engineered, artificial ECM proteins containing the RGD sequence (aRGD) but lacking the PHSRN synergy site. Furthermore, aRGD-mediated adhesion stimulates focal adhesion kinase (FAK) phosphorylation at Y397, an event that is indicative of mechanical coupling of integrin to the substratum and of promoting adhesion-dependent cell proliferation. Although both aRGD and the natural ECM protein fibronectin (FN) support cell spreading and share certain features of cell signaling, a significant, quantitative difference was identified by single-cell analysis of cell spreading and integrin expression. The threshold amount of integrin required for spreading on aRGD-coated substrata was approximately 10-fold greater than that required for FN-mediated spreading. We further demonstrate that this performance gap in mediating cell spreading may be narrowed by composite surfaces that present a surrogate HBD and aRGD.