Mixed Integrin-Specific Surfaces Synergistically Modulate Signal Transduction and Cell Proliferation

Reyes, Catherine D., Petrie, Timothy A., and García, Andrés J.
Woodruff School of Mechanical Engineering, Georgia Institute of Technology, Atlanta, GA 30332 USA

Introduction: Cell adhesion to the extracellular matrix (ECM) through cell-surface integrin receptors is essential to development, wound healing, and tissue remodeling, and therefore represents a central theme in the design of bioactive surfaces that successfully interface with the body. The objective of this study is to engineer bioactive hybrid surfaces that control cell function by mimicking integrin-ECM interactions. We target two specific integrins essential to differentiation in several cell systems – the type I collagen (COL-I) receptor $\alpha_2\beta_1$ and the fibronectin (FN) receptor $\alpha_5\beta_1$ – by tethering varying densities of a recombinant FN fragment and a collagen-mimetic peptide onto non-adhesive supports.

Methods: The $\alpha_2\beta_1$-specific peptide used in this study contains the GFOGER adhesion motif from COL-I ($GGYGGGPC(GPP)_5GFOGER(GPP)_5GPC$) [3,4]. The FN-mimetic ligand was a recombinant fragment of FN (FNIII7-10) that encompasses the PHSRN and RGD binding domains from the native protein that synergize to specifically target the $\alpha_5\beta_1$ integrin [5]. To develop model ECM-mimetic surfaces, biotinylated FNIII7-10 and GFOGER-peptide onto non-fouling, cell adhesion-resistant avidin substrates. To generate mixed surfaces, the two biotinylated ligands were added sequentially to avidin surfaces for 1 hr each. HT1080 cell adhesion to the mixed ligand surfaces was measured under serum-free conditions using a centrifugation assay [6]. These cells adhere to COL-I and FN via $\alpha_2\beta_1$ and $\alpha_5\beta_1$, respectively. Cell signaling properties of these surfaces were assessed with spot blotting for specific phosphorylation sites on focal adhesion kinase (FAK). Proliferation rate was measured by 12 hour BrdU incorporation and quantified by flow cytometry.

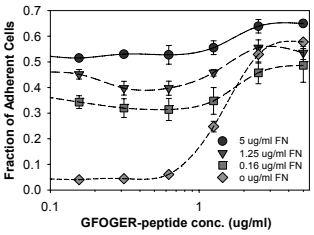

Fig. 1: HT1080 human fibrosarcoma adhesion on mixed surfaces. (1 hour cell adhesion, 12g centrifugation for 5 min.)

Results: Controlled densities of tethered biotinylated ligands were obtained by varying the coating solution concentration. Cell adhesion profiles on these surfaces (**Fig. 1**) correlated well with relative ligand densities reflected in ELISA data (not shown). For high FNIII7-10 concentrations, the adhesion profiles were insensitive to changes in GFOGER density at low densities but increased with high peptide densities. As FNIII7-10 concentration decreased, the tethered GFOGER recovered density-dependent increases in cell adhesion. When FNIII7-10 was eliminated from the surface, the adhesion profile again shifted to the right, demonstrating the reduced adhesive potential of the single-ligand functionalized surfaces compared with the mixed ligands. Antibodies against specific integrin subunits demonstrated the specificity of these engineered surfaces.

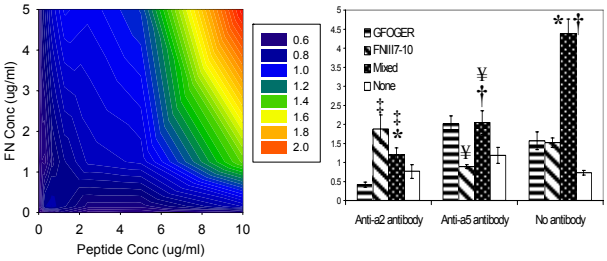

Fig. 2: Focal adhesion kinase activation on mixed ligand surfaces. **a**) Contour data represents Y397 phosphorylation on FAK. **b**) Anti-integrin antibody blocking of FAK activity. (1 hr adhesion, $p<0.05$)

We then examined whether these mixed surfaces trigger the activation of FAK, an intracellular signaling molecule central to integrin-mediated signal transduction and downstream differentiation pathways. A contour plot of the resultant data demonstrates a synergistic activation of FAK on the mixed surfaces (**Fig. 2a**). While FAK activation increased with both increasing GFOGER and increasing FN, there is a 3-fold increase in FAK activity at high levels of both ligands. Blocking either $\alpha_2\beta_1$ or $\alpha_5\beta_1$ (**Fig. 2b**) eliminated this synergistic effect, reducing FAK activity to levels equivalent to those triggered by the single ligands. To determine whether this synergy in intracellular signaling translates to a downstream cellular response, we examined proliferation on mixed and single ligand functionalized surfaces. **Fig. 3** demonstrates enhanced proliferation rate on the mixed surfaces, paralleling both the cell adhesion and the FAK activation data.

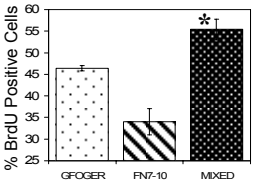

Fig. 3: Flow cytometry quantification of HT1080 cell proliferation using BrdU incorporation (BrdU added 24 hrs after cell seeding, incorporated for 12 hrs; * indicates different from GFOGER or FN7-10, $p<0.05$)

Conclusions: The wide range of controlled mixed ligand densities generated by this process demonstrates the feasibility of generating integrin-specific hybrid surfaces. The increased cell adhesion and synergistic activation of FAK underscore the advantage of specifically targeting more than one integrin implicated in a particular signaling pathway and downstream cellular effect. Proliferation rate results confirm that the enhanced signaling effects of mixed ligand surfaces translate to downstream cellular responses. In addition, increased levels of FAK have been correlated with increased differentiation of osteoblast cells. Screening of osteoblastic differentiation on these hybrid surfaces is currently being examined in vitro and in vivo. We expect that these ECM-mimetic surfaces can be used to identify optimal mixed ligand compositions that upregulate osteoblastic differentiation and matrix mineralization.

References: [1] G Xiao, *J.Biol.Chem.,* 273:32988-94 (1998); [2] AM Moursi et al, *J.Cell Sci.* 110, 2187-2196 (1997); [3] CD Reyes & AJ García *J.Biomed.Mater.Res* 65A:511-523 (2003); [4] CD Reyes & AJ García *J.Biomed.Mater.Res* 69A:591-600(2004); [5] SM Cutler & AJ García *Biomaterials* 24(10):1759-70 (2003). [6] CD Reyes & AJ García *J.Biomed.Mater.Res* 67A(1):328-33 (2003).

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