Fibronectin-mimetic Surfaces Directing $\alpha_5\beta_1$ Integrin-Mediated Adhesion, Signaling, and Proliferation

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Statement of Purpose: Cell adhesion to extracellular matrix proteins, such as fibronectin (FN), is primarily mediated by integrin receptors, which direct cell survival, growth, and differentiation [1]. Furthermore, we have shown that integrin binding specificity, particularly $\alpha_5\beta_1$, for adsorbed FN regulates surface-chemistry dependent osteoblast differentiation via modulation of downstream signaling pathways [2]. We have engineered a biomimetic model surface to specifically target the $\alpha_5\beta_1$ integrin. Since binding of $\alpha_5\beta_1$ to FN requires both the RGD and PHSRN synergy site [3], this surface presents a recombinant FN fragment ($FNIII_{7\text{-}10}$) which contains both RGD and PHSRN sites in the correct structural orientation.

Methods: Biotinylated $FNIII_{7\text{-}10}$ was expressed in *E. coli* and purified by affinity chromatography. Mixed self-assembled monolayers (SAMs) of alkanethiols on Au were used to present COOH anchoring groups within a non-fouling background. SAMs were prepared by immersing Au-coated substrates in 1.0 mM alkanethiol solution (19:1 HS-$[CH_2]_{11}$-$[OCH_2CH_2]_3OH$: HS-$[CH_2]_{11}$-$[OCH_2CH_2]_6OCH_2COOH$) for 4 hr. To tether ligands, SAM COOH groups were converted to active NHS-esters by incubating in 2 mM EDC and 5 mM NHS in 0.1 M MES (pH 6.0) to react with primary amines in the ligand [4]. $FNIII_{7\text{-}10}$ or GRGDS was tethered. Surface ligand density and cell binding was assessed by ELISA, adhesion centrifugation assays, and SPR. FAK Western blots and BrdU incorporation experiments with NIH3T3 and MC3T3-E1 cells were also performed to assess the functional activity of these surfaces.

Results/Discussion: Surface density of tethered $FNIII_{7\text{-}10}$ or RGD increased with coating concentration until saturation was reached. Tethered densities were 10 times greater than on background EG_3 and non-activated surfaces, demonstrating ligand tethering to the surface. Comparison of cell adhesion profiles using a centrifugation assay clearly demonstrated enhanced cell binding (> 10 fold) of the $FNIII_{7\text{-}10}$-tethered surface over the RGD-functionalized surface (Fig. 1). No cells adhered to surfaces without adhesive ligand.

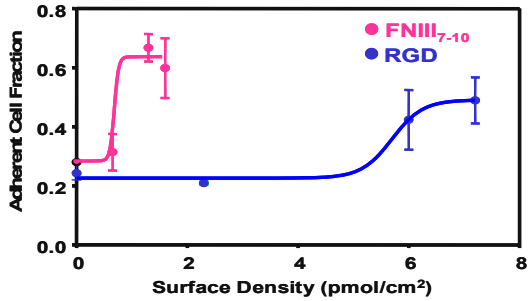

Fig. 1. Adherent cell adhesion profile of $FNIII_{7\text{-}10}$- and RGD-tethered surfaces, demonstrating enhanced adhesion for $FNIII_{7\text{-}10}$.

Blocking experiments with integrin-specific antibodies revealed that integrin $\alpha_5\beta_1$ provided the dominant adhesion mechanism to $FNIII_{7\text{-}10}$, while adhesion to RGD-functionalized surfaces was mediated by $\alpha_v\beta_3$ (Fig. 2). This result demonstrates the integrin binding specificity of these engineered surfaces.

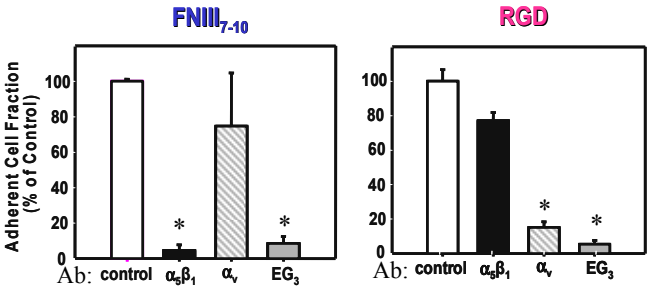

Fig. 2. Antibody blocking demonstrating integrin specificity.

In addition, FAK phosphorylation at Y397 was elevated on $FNIII_{7\text{-}10}$–tethered surfaces compared to RGD surfaces. Furthermore, cells on the $FNIII_{7\text{-}10}$ surface displayed enhanced proliferation rates over RGD-tethered surfaces (Fig. 3). This data demonstrate the enhanced adhesive and functional activities of this fibronectin-mimetic surface compared to RGD-functionalized substrates.

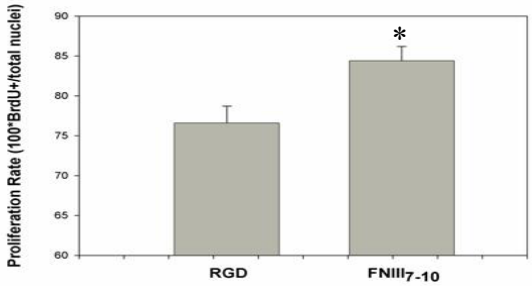

Fig.3: Proliferation rate of MC3T3-E1 cells on engineered surfaces after 24 hr adhesion (10 hr BrdU incorporation) ($p<0.024$).

Conclusions: We demonstrated that tethering of a FN recombinant fragment onto a synthetic support yields a bioadhesive surface specific for integrin $\alpha_5\beta_1$. This FN-mimetic surface exhibited enhanced adhesive activity, FAK activation, and proliferation rates compared to RGD-tethered substrates. This biomolecular strategy may provide a robust approach to engineer integrin binding specificity and control cellular signaling pathways. Current studies focus on the ability of this surface to support osteoblastic differentiation.

References : [1] RO Hynes, *Cell* 110, 673-87 (2002); [2] BG Keselowsky et al., PNAS 102 :17 5953-57; [3] AJ García et al. *Biochemistry* 41, 9063-69 (2002); [4] JR Capadona et al, *Adv Mater* (in press).

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