Recombinant Multimeric Integrin Ligands to Convey Integrin Specificity and Clustering

Timothy A. Petrie[1,3], Andrés J. García[1,3]
[1] Woodruff School of Mechanical Engineering; [2]Petit Institute for Bioengineering and Bioscience;
Georgia Institute of Technology, Atlanta GA 30332

Introduction: Recent studies have shown that integrin binding specificity can direct specific cell functional pathways including proliferation and differentiation [1]. Moreover, we have shown that a recombinant fragment of FN ($FNIII_{7-10}$) incorporating both the RGD and PHSRN synergy sites displays $\alpha_5\beta_1$ binding specificity and enhanced adhesive activities [2]. In addition to binding specificity, biophysical cues such as ligand spatial arrangement and clustering of integrins modulate cell adhesive responses [3]. To elucidate how integrin clustering regulates cell responses to biomaterials, we are engineering FN-mimetic interfaces presenting multi-valent ligands. We are using recombinant multimeric constructs presenting one, two, three, and five copies of the $FNIII_{7-10}$ integrin-binding domain of FN [4]. A major advantage of this system is that we can precisely control the valency of the ligand, instead of relying on 'statistical average' values as is typically done. We demonstrate that these multimeric ligands support robust $\alpha_5\beta_1$ binding and integrin-mediated cell adhesion.

Materials and Methods: Recombinant multimeric $FNIII_{7-10}$ proteins were expressed in BL21 cells and purified via anion-exchange chromatography [4]. The adhesion activity and integrin binding specificity of these constructs was assessed by a centrifugation adhesion assay in the presence of integrin subunit-blocking antibodies. Integrin binding was evaluated in a real-time binding assay using purified components. Purified integrin $\alpha_5\beta_1$ (Chemicon) was flowed (3 µl/min, 6 min) over Au chips presenting equimolar densities of adsorbed multimeric ligands and the amount of bound integrin measured by SPR.

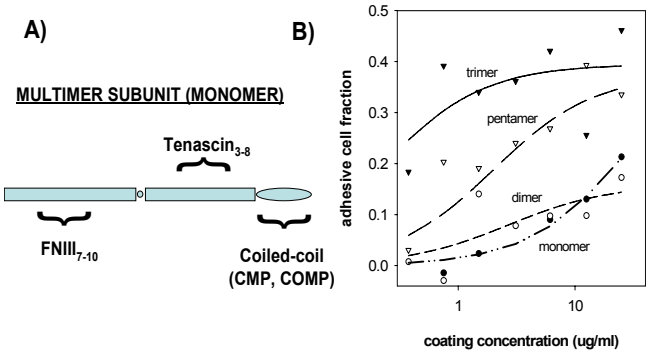

Fig.1: A) Schematic of multimeric $FNIII_{7-10}$ ligand. $FNIII_{7-10}$ is connected to a particular coiled-coil region which allows dimeric, trimeric, or pentameric assembly, depending on the exact coiled-coil region involved. B) Cell adhesion as a function of ligand concentration for multimeric ligands.

Results and Discussion: We are using monomeric and multimeric (dimer, trimer, pentamer) FN constructs to convey integrin clustering. The ligands consist of $FNIII_{7-10}$ at the N-terminus, followed by a spacer arm comprising the FN-III domains 3-8 from tenascin, followed by an oligomerization sequence (K6 peptide for dimer, CMP for trimer, COMP for pentamer) (Fig.1A). Western blotting (under non-reducing conditions) verified successful expression and assembly of multimers. Blots demonstrated expected increases in MW with multimer valency (data not shown). ELISA measurements revealed that the availability of the integrin binding site as a function of coating concentration was equivalent among multimeric FN constructs. Cell adhesion to engineered ligands as a function of ligand concentration was measured by a centrifugation assay. For all ligands, cell adhesion increased with available ligand. Moreover, adhesion levels were enhanced on the trimeric and pentameric constructs compared to the monomer (Fig 1B). These results demonstrate synergistic effects for clustered ligand presentation. Experiments with integrin-specific antibodies demonstrated that adhesion to these ligands was mediated by the $\alpha_5\beta_1$ integrin (data not shown).

To investigate the ability of these FN-mimetic constructs to support integrin binding, $\alpha_5\beta_1$ integrin was flowed in integrin activating conditions (PBS + 1mM Mn^{+2}) over equimolar densities of multimers on SPR chips. The amount of bound integrin, determined from the mass of integrin remaining after a short PBS wash, varied among each multimer type (Fig 2), increasing linearly with multimer valency (R^2=0.99). This data demonstrates that the multimers support integrin binding in the predicted manner, and, further, that these integrin binding sites are indeed accessible.

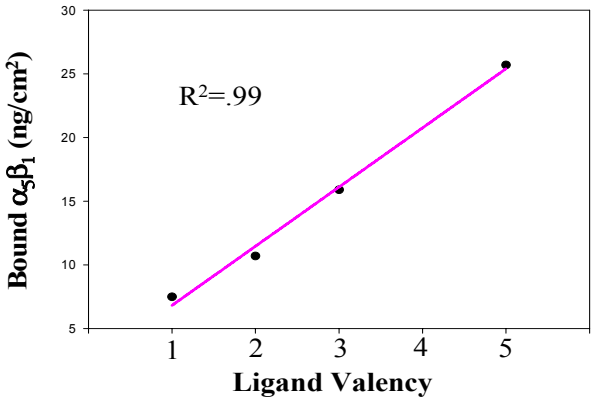

Fig.2: A) Integrin binding to multimeric ligands analyzed by SPR. Integrin was flowed over equimolar (3 pmol/cm^2) ligand surfaces. Bound integrin correlated directly with number of multimer valency.

Conclusions: We have expressed FN-mimetic multimeric constructs to promote integrin clustering. These ligands support $\alpha_5\beta_1$ binding with the number of bound integrins increasing linearly with binding site repeat. We are currently evaluating the cell adhesion strength and signaling on these multimeric FN-mimetic constructs.

References : [1] Keselowsky et al., *PNAS*, 102, 5953-57 (2005); [2] Petrie et al. *Biomaterials*, 27(31):5459-70 (2006); [3] Koo et al., *J Cell Sci.*, 115:1423-33 (2002); [4] Coussen et al. , *J Cell Sci,* 115:2581-90 (2002).

Acknowledgements: This work was supported by NIH (R01 EB-004496) & NSF CAREER (BES-0093226), with additional support from Medtronic Foundation. Multimeric construct cDNA was generously provided by Harold Erickson (Duke Univ.).