Supporting Information

Using Self-Assembled Monolayers to Model Cell Adhesion to the 9th and 10th Type III Domains of Fibronectin

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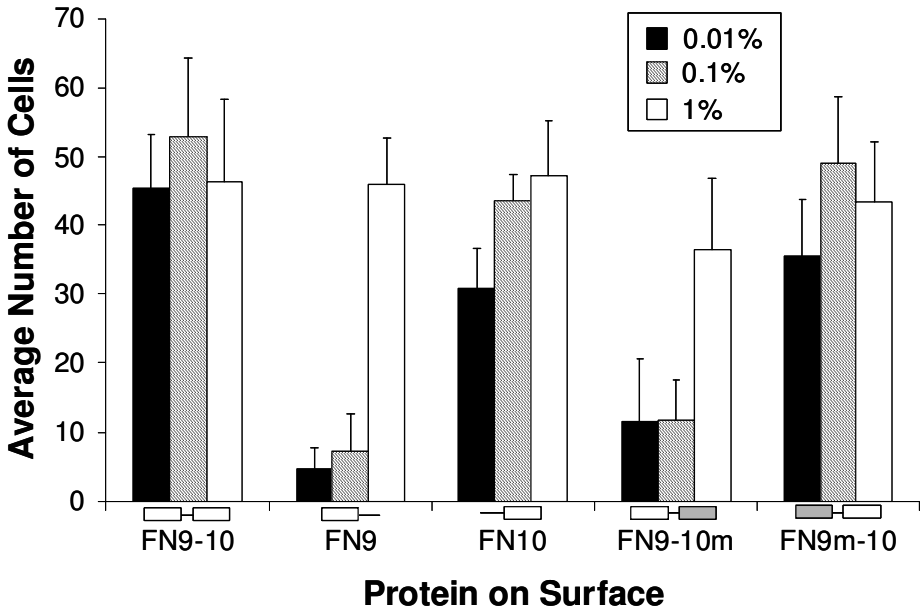
The gene splicing by overlap extension polymerase chain reaction (SOEing PCR) technique was used to create fusions between the protein cutinase and the fibronectin domains. All of the primers (Sigma Genosys) used can be found in Supporting Table S1. To make the construct Cut-FN9, primers A and B were used to amplify the cutinase gene from the previously prepared vector pCut22b [Hodneland et al. *PNAS* **2002,** *99*, 5048-5052], and primers C and D were used to amplify the 9th type III domain from the plasmid FNIII7-10(pET11b) (courtesy of Dr. Harold Erickson [Leahy et al. *Cell* **1996,** *84*, 155-166]). 30 cycles of the individual PCR reactions described above were run using Pfu Turbo DNA polymerase (Stratagene, La Jolla, CA). Next, 25 μL of the cutinase reaction and 25 μL of the FN9 reaction were combined along with an additional 1 μL polymerase for a second round of 7 PCR cycles. Then, 1 μL of the combined PCR reaction was removed for a third PCR reaction setup using primers A and D for 30 cycles. The same general procedure was used to make the other constructs, using the respective primers A-D designated in Table S1 for each individual construct. To make the mutant constructs Cut-FN9-10m and Cut-FN9m-10, the Cut-FN9-10 construct was used as the PCR template. For the Cut-FN9m-10m construct, Cut-FN9-10m was the template, and the primers for Cut-FN9m-10 were used. The final products were cloned into the vector pET22b (Novagen, Gibbstown, NJ) between the NcoI and HindIII sites. Also, because expression of soluble Cut-FN9 was extremely low in pET22b, that product was also cloned into pET28a, which gave more satisfactory protein yields. All final products were confirmed by DNA sequencing.

Cut-FN9-10	5'-TTTTCCATGGGCCTGCCTACTTCTAACCCT-3'	A
	5'-TTTTATCAAGACCGGATCCTCCTCCAGCAGAACC-3'	B
	5'-TTTTCTGGAGGAGGATCCGGTCTTGATTCCCCAACTGGCATT-3'	C
	5'-TTTTAAGCTTATGTTCGGTAATTAATGGAAATTGGCT-3'	D
Cut-FN9	5'-TTTTCCATGGGCCTGCCTACTTCTAACCCT-3'	A
	5'-TTTTATCAAGACCGGATCCTCCTCCAGCAGAACC-3'	B
	5'-TTTTCTGGAGGAGGATCCGGTCTTGATTCCCCAACTGGCATT-3'	C
	5'-TTTTAAGCTTATGTTGATTGTTGGCCAATCAATAAGGG-3'	D
Cut-FN10	5'-TTTTCCATGGGCCTGCCTACTTCTAACCCT-3'	A
	5'-TTTTATCAAGACCGGATCCTCCTCCAGCAGAACC-3'	B
	5'-TTTTCTGGAGGAGGATCCGTTTCTGATGTTCCGAGGGACCT-3'	C
	5'-TTTTAAGCTTATGTTCGGTAATTAATGGAAATTGGCT-3'	D
Cut-FN9-10m	5'-TTTTCCATGGGCCTGCCTACTTCTAACCCT-3'	A
	5'-TTTTGCGGGGCTTCCGTCACGGCCAGTGACAGCATACACAGTGATGGTA-3'	B
	5'-TTTTGGCCGTGACGGAAGCCCCGCAAGCAGCAAGCCAATTTC-3'	C
	5'-TTTTAAGCTTATGTTCGGTAATTAATGGAAATTGGCT-3'	D
Cut-FN9m-10	5'-TTTTCCATGGGCCTGCCTACTTCTAACCCT-3'	A
	5'-TTTTGTGATGGAATTAGAGGGCCGGTGCACCCGATCTTCTCGAGGTCTCC-3'	B
	5'-TTTTCACCGGCCCTCTAATTCCATCACCCTCACCAACCTCAC-3'	C
	5'-TTTTAAGCTTATGTTCGGTAATTAATGGAAATTGGCT-3'	D

Supporting Table S1. Primers used with the SOEing PCR technique to create the cutinase-fibronectin domain fusion proteins.



Supporting Figure S1. Density dependence of cell adhesion to the protein substrates. The protein substrates containing the intact RGD peptide (FN9-10, FN10, and FN9m-10) are able to sustain cell adhesion at low densities. However, those substrates lacking RGD (FN9 and FN9-10m) display attachment at a density of 1% protein.