Electronic Supplementary Material (ESI) for Integrative Biology.

ELECTRONIC SUPPLEMENTARY INFORMATION for

Single molecular force across single integrins dictates cell spreading

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Experimental Procedures

Passivated surface preparation

Glass coverslip surfaces were functionalized and passivated by mixing biotinylated PEG of different molecular weights (0.4kD, 5kD, 10kD, 20kD) with mPEG (5kD) molecules at a ratio of 1:5. The surface passivation technique is described elsewhere in details[1]. In short, cleaned glass coverslip surfaces were aminosilanized and the amines were reacted with PEG-N-hydroxysuccinimide (NHS) ester: mPEG-SVA (MW 5kD, Laysan Bio, Inc) and biotin-PEG (0.4kD, Thermo Fisher Scientific Inc.; and 5kD, 10kD, and 20kD, Laysan Bio, Inc). Passivated surfaces were incubated with NeutraAvidin (200 µg/ml, Thermo Fisher Scientific Inc.) in PBS for 20 mins. The coverslips were washed by PBS twice and one time with dH_2O and air dried. 3 µl volume spots of 1 µM RGD-TGTs of different tension tolerance were incubated on the surfaces. These spots were further incubated at 4° C for 30 minute and then washed by PBS to remove unbound TGTs. Thus, the TGT engineered surfaces were ready for cell adhesion assay.

Extended freely-jointed chain (xFJC) model of PEG

The force-extension curves and the corresponding stiffness were also calculated by the extended freely jointed chain model[2].

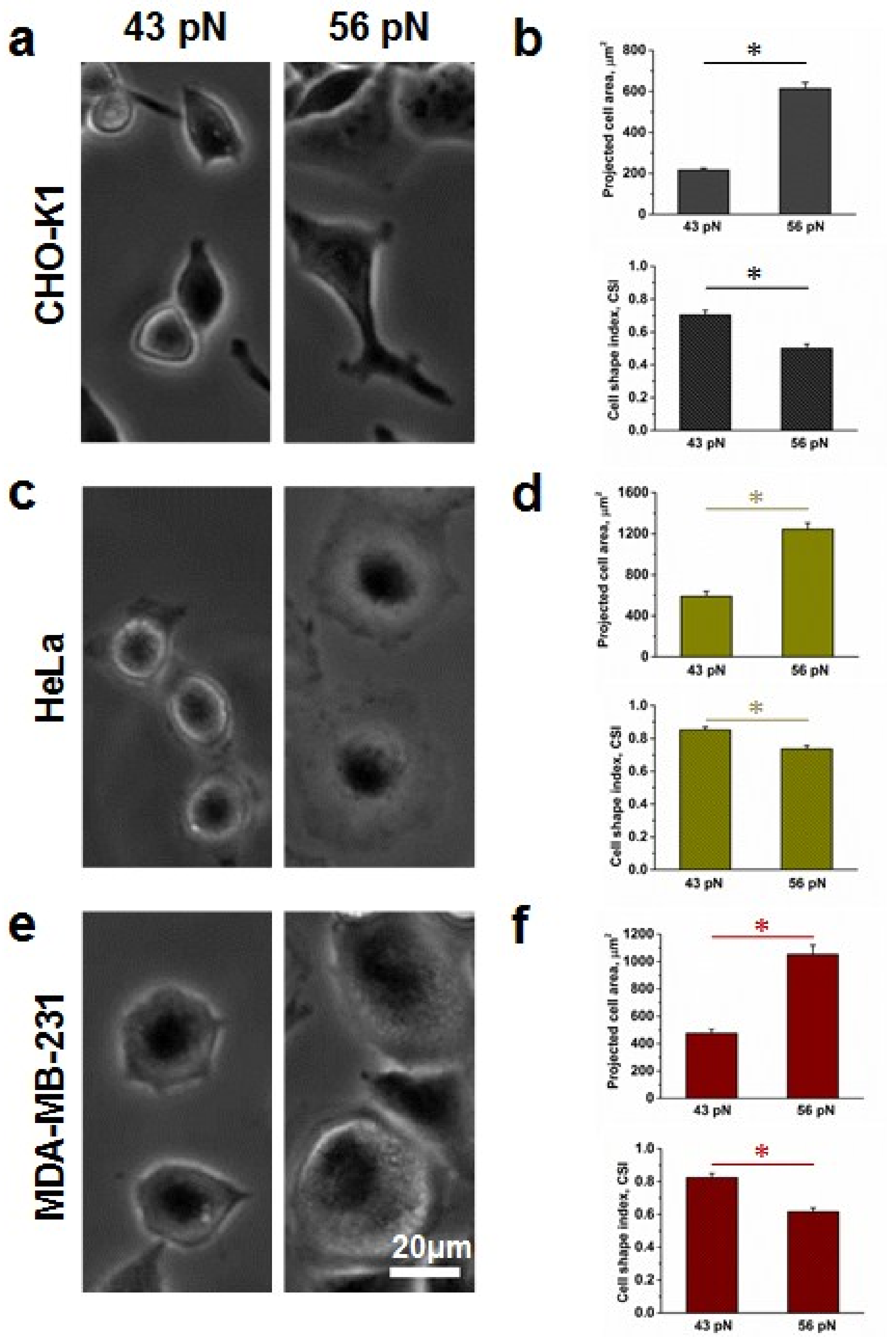
$$L(F) = L_c(F)\left(\coth\left(\frac{FL_k}{k_BT}\right) - \frac{k_BT}{FL_k}\right) + \frac{N_sF}{K_s}$$

The number of monomer units N_s corresponding to PEGs with different molecular weights (0.4k, 5k, 10k, 20k) are 12, 146, 293 and 587. The Kuhn length L_k of PEG is 0.7 nm[2]. The contour length L_c is calculated as the product of the number of monomer and Kuhn length. The segment elasticity K_s is 150 N/m/monomer[2].

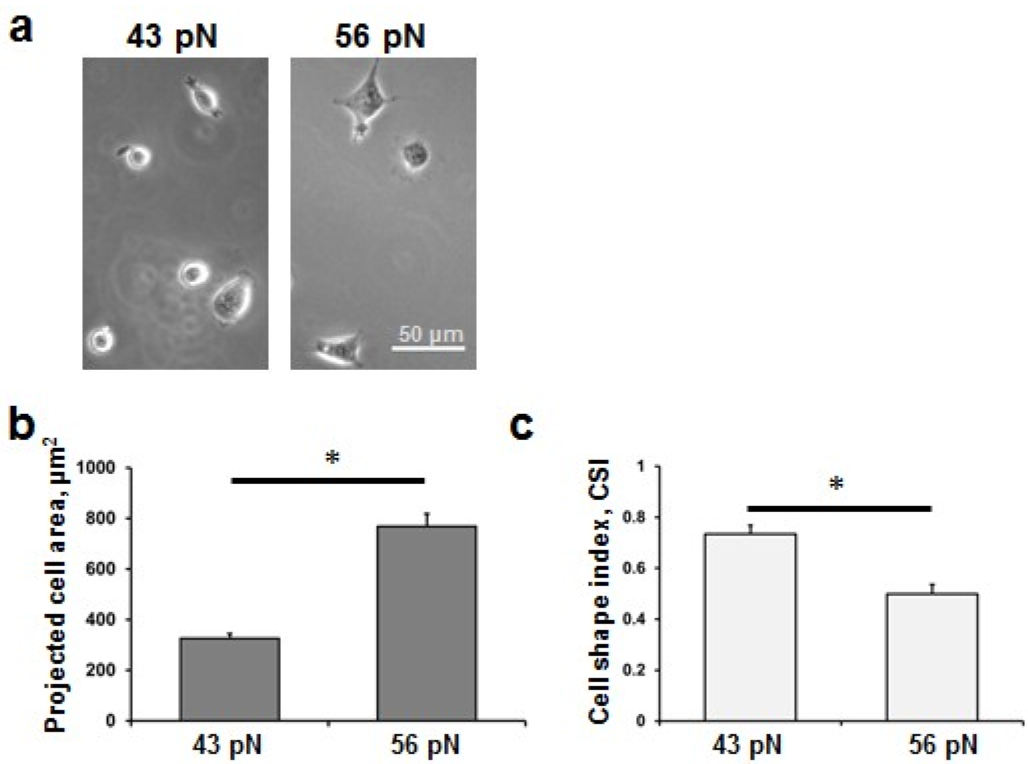
Fabrication of Polyacrylamide substrates

Polyacrylamide gel substrates were prepared as described before[3]. The polyacrylamide gel substrates with elastic Young's modulus of 1.0 kPa (0.1% bis-acrylamide, 3% polyacrylamide) and 8 kPa (0.3% bis-acrylamide, 5% polyacrylamide) were used in this study[4-6].

Supplementary Figures



Supplementary Figure 1. Cell spreading area increases with increasing molecular tension tolerances. (**a**, **c**, **e**) Representative images of CHO-K1, HeLa, and MDA-MB-231 cells on 43 and 56 pN surfaces. (**b**, **d**, **f**) Projected cell area of CHO-K1, HeLa, and MDA-MB-231 cells increases while CSI values decreases with increasing tension tolerances. For CHO-K1 cells, $n=26$ and 27 for 43 pN and 56 pN surfaces. For HeLa cells, $n=32$ and 36 for 43 pN and 56 pN surfaces. For MDA-MB-231 cells, $n=41$ and 47 for 43 pN and 56 pN surfaces. Data represents mean± s.e.m. $^{*}p < 0.001$.



Supplementary Figure 2. Molecular tension tolerance dependent cell spreading in mouse embryonic fibroblasts (MEFs). (**a**) Representative images MEFs on 43 and 56 pN surfaces. (**b**-**c**) Projected cell area of MEFs increases while CSI values decreases with increasing tension tolerances (n=23 for both 43 pN and 56 pN surfaces). Data represent mean± s.e.m. *p<0.001

References

1. R. Roy, S. Hohng and T. Ha, *Nat Methods*, 2008, 5, 507-516.
2. F. Oesterhelt, M. Rief and H. E. Gaub, *New J Phys*, 1999, 1.
3. R. J. Pelham, Jr. and Y. Wang, *Proc Natl Acad Sci U S A*, 1997, 94, 13661-13665.
4. T. Yeung, P. C. Georges, L. A. Flanagan, B. Marg, M. Ortiz, M. Funaki, N. Zahir, W. Ming, V. Weaver and P. A. Janmey, *Cell Motil Cytoskeleton*, 2005, 60, 24-34.
5. A. Engler, L. Bacakova, C. Newman, A. Hategan, M. Griffin and D. Discher, *Biophys J*, 2004, 86, 617-628.
6. J. R. Tse and A. J. Engler, *Curr Protoc Cell Biol*, 2010, Chapter 10, Unit 10 16.