# SYNERGISTIC REGULATION OF CELL FUNCTIONS BY MATRIX RIGIDITY AND ADHESIVE PATTERN USING AN ELASTOMERIC MICROPOST ARRAY SYSTEM

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## ABSTRACT

Interactions between cells and their surrounding extracellular matrix (ECM) play a critical role in regulating cell function [1-2]. Recent studies have primarily focused on mechanotransduction by cells to sense and respond to matrix rigidity. Through these studies, it becomes clear that mechanotransduction is likely mediated by the complex interplay between the mechanosensitive focal adhesion (FA) signaling and actomyosin-mediated cytoskeletal contraction [3-4]. However, the impact of other ECM features such as adhesive ECM patterns on cellular sensing of ECM rigidity is still elusive and not well characterized. Here, we performed a systematic study to investigate the independent effect of adhesive ECM pattern on matrix rigidity-mediated mechanoresponsive behaviors of different adherent cells, including NIH/3T3 fibroblasts and human umbilical vein endothelial cells (HUVECs), by using a novel library of microfabricated synthetic elastomeric polydimethylsiloxane (PDMS) micropost arrays [5-6].

**KEYWORDS:** Matrix Rigidity, Mechanosensing, Mechanotransduction, Adhesive ECM Pattern

## INTRODUCTION

There is mounting evidence indicating that the physical signals in the cell microenvironment including matrix rigidity can profoundly affect cell-ECM interactions to modulate downstream cell functions, such as cell adhesion, migration, proliferation, and even stem cell differentiation [7-10]. One primary molecular mechanism of rigidity sensing is through the actomyosin mediated cytoskeletal contraction, which promotes clustering of integrins and the accumulation of FA proteins [11]. The cytoskeletal contractile forces directly regulate FA formation and morphogenesis and further activate several kinases in FAs (such as FAK and Src) involved in regulation of cell function [12-13]. Recent investigations have further identified different FA proteins such as talin [14], vinculin [15] and p130-CAS [16] as mechanosensors to mediate downstream signaling pathways that could control cytoplasmic $Ca^{2+}$ permeability and thus directly regulate cytoskeletal contraction [17]. Although a good amount of molecular details for the mechanosensing process have been revealed through recent studies , there are still unanswered question in the field, such as those involved in the long-term mechanical regulation of stem cell differentiation. In addition, the impact of other ECM features such as adhesive ECM patterns on cellular sensing of ECM rigidity is still elusive and not well characterized. Given that molecular scale surface properties of the ECM could dramatically regulate cell behaviors [18], we hypothesize that matrix rigidity and adhesive ECM patterns might synergistically and collectively interplay to regulate the cell-ECM interactions and thus the downstream cellular behaviors.
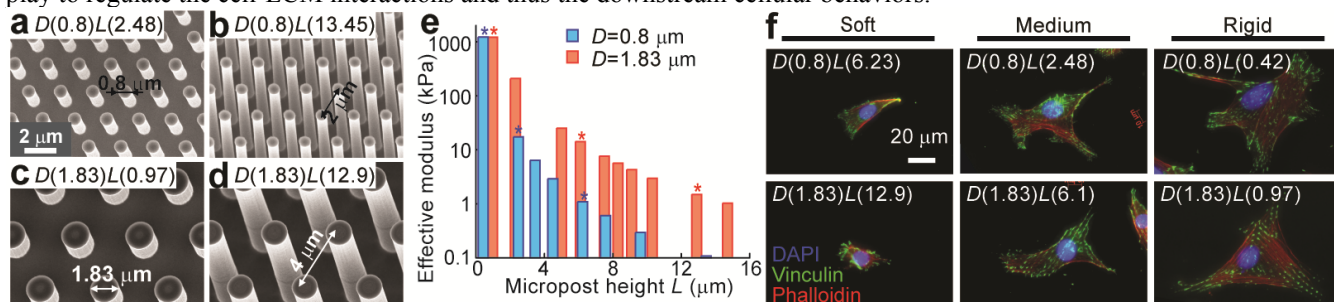


*Figure 1: Elastomeric PDMS micropost arrays to engineer substrate rigidity and adhesive ECM patterns. (a-d) Scanning electron images (SEM) of silicon micropost array masters. (e) Dependence of effective modulus $E_{eff}$ of PDMS micropost on micropost height L and micropost diameter D. Asterisks (\*) mark the major PDMS micropost arrays with comparable $E_{eff}$ that were selected for comparative studies in this work. (f) Representative staining images of single NIH/3T3 cells plated on paired PDMS micropost arrays of similar effective moduli but different rigidities.*

## RESULTS AND DISCUSSION

To investigate the independent effect of adhesive ECM pattern on matrix rigidity-mediated mechanoresponsive behaviors, we designed and fabricated two sets of hexagonally spaced PDMS micropost arrays with different post diameters, both sets covering a broad range of substrate rigidities with modulated post heights (Fig. 1a-e). Different PDMS micropost arrays were deliberately selected from each set and paired together based on their comparable effective moduli $E_{eff}$ (Fig. 1e). Using

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these PDMS arrays, we performed a comparative study to examine independent effects of substrate rigidity and adhesive ECM pattern on mechanoresponsive behaviors of adherent cells. Our results showed that the mechanoresponsive behaviors of cells, including cell spreading, FA formation, and cell proliferation were strongly mediated by substrate rigidity (Fig. 2). In the test range of rigidity, we also noticed a significant and independent effect of the adhesive ECM pattern on these mechanoresponsive cell behaviors. In general, the adhesive ECM pattern for the *D(0.8)* micropost arrays elicited greater cell spreading, FA formation, and proliferation than the pattern for the *D(1.83)* micropost arrays. The effect of adhesive ECM pattern on the mechanoresponsive cell behaviors was cell-type specific and also dependent on the specific rigidity range.
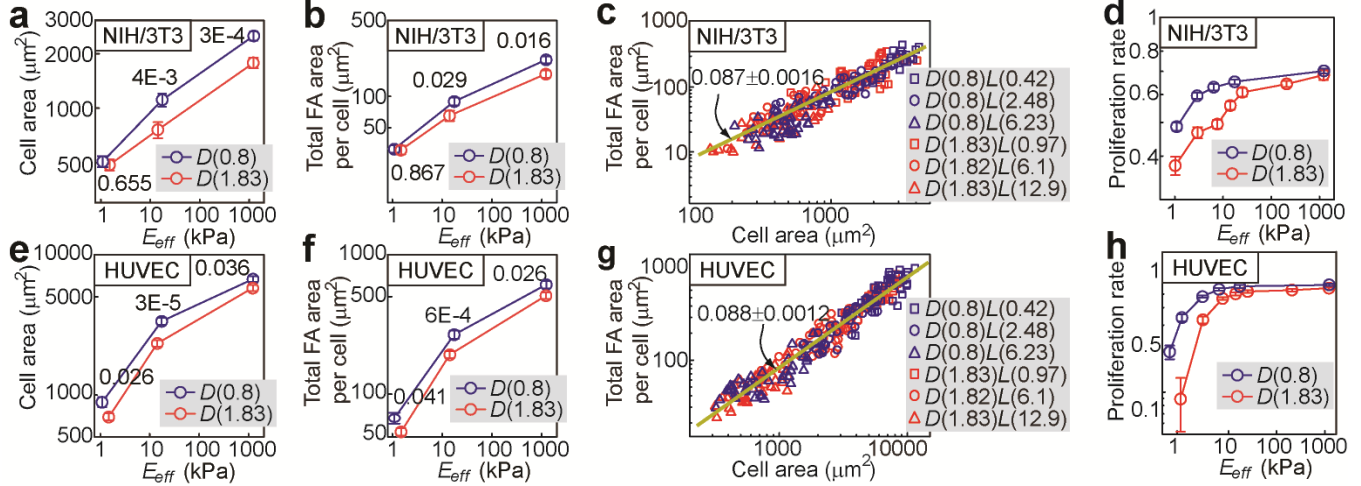


*Figure 2: Quantitative and comparative analysis of effects of matrix rigidity and adhesive ECM pattern on cell morphology (a&e), FA formation (b&f), and cell proliferation (d&h). Correlative study was also performed for total FA area per cell and cell spread area in (c) and (g). Data were collected and compared from single NIH/3T3 (a-d) and single HUVECs (e-h) plated on PDMS micropost arrays of different geometries and rigidities. The D(0.8) micropost arrays were symbolized in blue, and the D(1.83) micropost arrays were symbolized in red.*
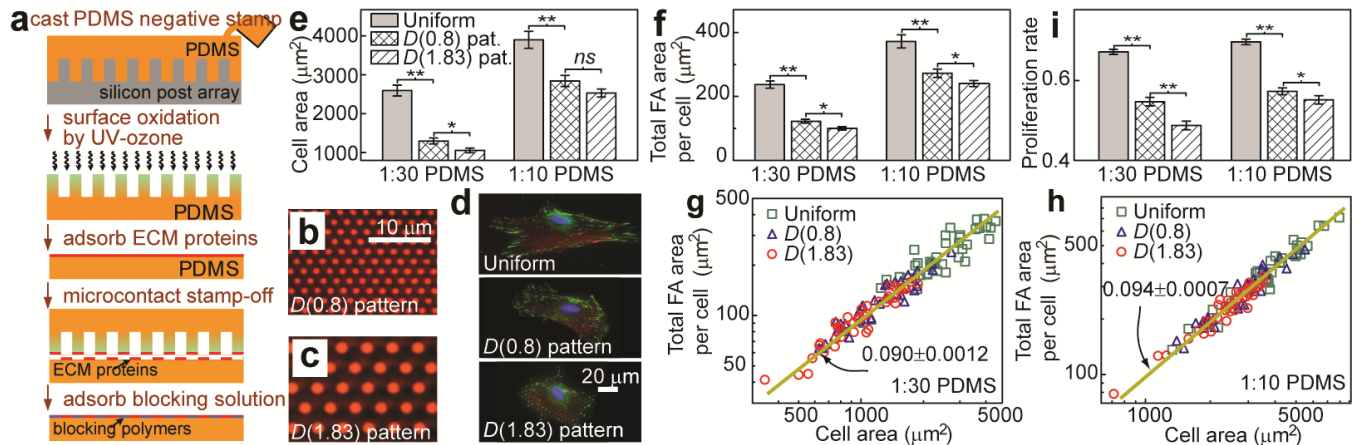


*Figure 3: Control experiments on flat PDMS substrates coated with different adhesive ECM patterns. (a) Schematic of microcontact stamp-off. (b&c) Fluorescence images of flat PDMS substrates patterned with adhesive ECM islands. (d) Representative staining images of single NIH/3T3 cells plated on flat PDMS substrates coated with uniform ECM molecules (top) or adhesive ECM islands (middle and bottom). (e-i) Quantitative and correlative analysis of the effect of matrix rigidity and adhesive ECM patterns on flat PDMS substrates on cell morphology, FA formation, and proliferation of NIH/3T3 cells. Correlative study was also performed for total FA area per cell and cell spread area in (g) and (h).*

We further generated flat and continuous PDMS substrates patterned with adhesive ECM islands using microcontact stamp-off (Fig. 3a). Patterned ECM islands on flat PDMS surfaces shared the same geometries with the tops of the PDMS micropost arrays (Fig. 3b&c). Uniformly coated continuous PDMS substrate was also examined to gauge the effect of available adhesive area on mechanosensitive cell behaviors. Our results in Fig. 3 showed that the adhesive ECM pattern could significantly influence cell spreading, FA formation and proliferation on flat PDMS substrates (Fig. 3e-i). It appeared that smaller and denser adhesive islands facilitated rigidity sensing and as such resulted in enhanced mechanoresponsive cellular

behaviors. Further, we had observed a strong linear correlation between cell spread area and total FA area per cell, regardless of matrix rigidity or adhesive ECM pattern (Fig. 2c&g and Fig. 3g&h), which highlighted the complex functional interplay between mechanosensitive FA formation and cell morphology in mediating cell-ECM interactions and rigidity sensing.

## CONCLUSIONS

In summary, we applied a novel library of PDMS micropost arrays to investigate effect of adhesive ECM pattern on mechanoresponsive cellular behaviors. Our data in Fig. 2 and Fig. 3 suggested that mechanosensitive behaviors of adherent cells was mediated by both matrix rigidity and presentation of adhesive ECM proteins, and their synergistic regulation of cell-ECM interactions could be cell-type specific and could also depend on the specific rigidity range of the matrix the cells adhere to. Our comparative and correlative studies also strongly indicated that cell shape and FA structures were tightly coupled mechanoresponsive systems involved in transducing physical signals in cell microenvironments into intracellular responses. Our work here might prove important for designing synthetic biomaterials for directed cell behaviors, critical for tissue engineering and regenerative medicine.

## REFERENCES

[1] Geiger, B., J.P. Spatz, and A.D. Bershadsky, *Environmental sensing through focal adhesions.* Nat Rev Mol Cell Biol, 2009. 10(1): p. 21-33.

[2] Schwartz, M.A. and D.W. DeSimone, *Cell adhesion receptors in mechanotransduction.* Current Opinion in Cell Biology, 2008. 20(5): p. 551-556.

[3] Chen, C.S., *Mechanotransduction - a field pulling together?* Journal of Cell Science, 2008. 121(20): p. 3285-3292.

[4] Peyton, S., et al., *The emergence of ECM mechanics and cytoskeletal tension as important regulators of cell function.* Cell Biochemistry and Biophysics, 2007. 47(2): p. 300-320.

[5] Fu, J., et al., *Assaying stem cell mechanobiology on microfabricated elastomeric substrates with geometrically modulated rigidity.* Nature Protocols, 2011. 6: p. 187-213.

[6] Fu, J., et al., *Mechanical regulation of cell function using geometrically modulated elastomeric substrates.* Nature Methods, 2010. 7: p. 733-736.

[7] Discher, D.E., P. Janmey, and Y.-l. Wang, *Tissue Cells Feel and Respond to the Stiffness of Their Substrate.* Science, 2005. 310(5751): p. 1139-1143.

[8] Vogel, V. and M. Sheetz, *Local force and geometry sensing regulate cell functions.* Nat Rev Mol Cell Biol, 2006. 7(4): p. 265-75.

[9] Pelham, R.J. and Y.-l. Wang, *Cell locomotion and focal adhesions are regulated by substrate flexibility.* Proceedings of the National Academy of Sciences of the United States of America, 1997. 94(25): p. 13661-13665.

[10] Engler, A.J., et al., *Matrix Elasticity Directs Stem Cell Lineage Specification.* Cell, 2006. 126(4): p. 677-689.

[11] Burridge, K., M. Chrzanowska-Wodnicka, and C. Zhong, *Focal adhesion assembly.* Trends in cell biology, 1997. 7(9): p. 342-347.

[12] Hoffman, B.D., C. Grashoff, and M.A. Schwartz, *Dynamic molecular processes mediate cellular mechanotransduction.* Nature, 2011. 475(7356): p. 316-323.

[13] Geiger, B., J.P. Spatz, and A.D. Bershadsky, *Environmental sensing through focal adhesions.* Nature Reviews Molecular Cell Biology, 2009. 10(1): p. 21-33.

[14] del Rio, A., et al., *Stretching single talin rod molecules activates vinculin binding.* Science, 2009. 323(5914): p. 638-41.

[15] Grashoff, C., et al., *Measuring mechanical tension across vinculin reveals regulation of focal adhesion dynamics.* Nature, 2010. 466(7303): p. 263-266.

[16] Sawada, Y., et al., *Force sensing by mechanical extension of the Src family kinase substrate p130Cas.* Cell, 2006. 127(5): p. 1015-1026.

[17] Kobayashi, T. and M. Sokabe, *Sensing substrate rigidity by mechanosensitive ion channels with stress fibers and focal adhesions.* Curr Opin Cell Biol, 2010. 22(5): p. 669-76.

[18] Keselowsky, B.G., D.M. Collard, and A.J. Garcia, *Integrin binding specificity regulates biomaterial surface chemistry effects on cell differentiation.* Proc Natl Acad Sci U S A, 2005. 102(17): p. 5953-7.

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