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LOCAL ECM STIFFNESS MODULATES EPITHELIAL CELL RESPONSE TO MICROPATTERNS

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INTRODUCTION

Cells respond to substrate mechanical confinements with changes in morphology, proliferation, and motility. It is widely observed that cells grown on narrow stripes stretch themselves along the orientation of the stripes [1-3]. Such cells develop thick stress fibers along the stripes suggesting a reinforcement of the cytoskeleton in response to the geometrical constraints [4]. Nucleus of the cell is connected to the surrounding cytoskeleton via linker of nucleoskeleton and cytoskeleton complex. The alteration of mechanical environments around the nucleus can also trigger the change in nucleus shape. In human endothelial cells, the mechanical confinement caused nuclear deformation is attributed to the enhanced apical stress fibers that press the nucleus envelope [5-7]. However, in epithelial cells, shear stress disassembles stress fibers, the nucleus shrinks through Piezo1 mediated Ca^{2+} signaling [8]. Thus, the cells utilize complex force transduction mechanisms in response to external mechanical signals.

In this study, we have created fibronectin stripe patterns on substrates of varying stiffness and investigated the roles of substrate stiffness on cell response to mechanical confinements in MDCK cells. To assess the involvement of Piezo1, we knockdown Piezo1 with miRNA. Our results show that cells grown on soft patterns are significantly longer than cells on stiffer patterns. The cell elongation coincides with nuclear deformation. Knockdown of mechanosensitive Piezo1 protein significantly reduced the elongation on stiffer substrates but has minimal effect in cells on soft substrates. This result shows that increasing tension in stress fibers is not required for MDCK cell expansion on soft substrate, thus, different mechanisms are involved.

METHODS

PDMS substrates of various stiffness were made using Sylgard 184 and Sylgard 527. Four stiffness were tested, hard substrate (184 sylgard 10:1, stiffness ~ 1 MPa), semi-soft substrate (184 Sylgard 40:1, stiffness ~ 25 KPa) and soft substrate (527 Sylgard 1:0.8 ~1 KPa), and glass substrate (stiffness >1 GPa). To create micropatterns on the soft substrate, a reverse microcontact printing technique was used. Briefly, the substrate was plasma treated, followed by coating with monolayer of Fibronectin. The PDMS stamp (10 μm feature, 6 μm grove) was plasma treated for 30 s and then placed in contact with the substrate for 1 hr. The substrate was then treated with Pluronics F-127 for 1 hr to block non-selective cell attachment.

Prior to experiments, MDCK cells were seeded onto the patterned substrates and placed in incubator for 150 min. Following live-cell imaging, the cells were fixed and stained with Phalloidin Alexa Fluor 568 and Hoechst 33342 dye. Images were captured with a Zeiss inverted microscope with a CCD camera.

Statistical analysis used two-sample t-test. Values of $p < 0.005$ were considered statistically significant.

RESULTS

Substrate stiffness regulates cell elongation on micropatterns

To test the effect of substrate stiffness on MDCK cell response to narrow fibronectin stripes, we patterned micro-stripes (6 μm wide and 10 μm spacing) on substrates with various stiffness, namely, glass, hard, semi-soft, and soft. Cells have shown elongation on fibronectin stripes on all substrates. Interestingly, the cell elongation on soft substrate is significantly longer than stiffer substrates (Fig. 1a). The typical cells linear expansion on soft substrate are ~2 times longer than cells on glass. Cells showed similar expansions on semi-soft and soft substrates. The aspect ratio of cells on different substrates were analyzed using previously developed method [3]. Statistical analysis shows that the difference between soft and stiffer substrates is significant (Fig. 1b). Immunostaining F-actin with phalloidin showed that cells exhibit stronger actin stress fibers on the hard substrates compared with soft substrates. This suggests that stress fiber generated tension is not responsible for MDCK cell expansion on the soft substrates.

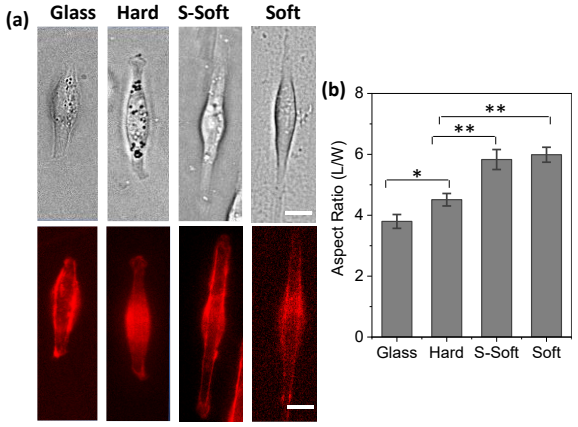

Figure 1: MDCK cell elongation on micropatterns with various stiffness. (a) Cells on soft stripes show maximal elongation but weaker stress fibers. **(b)** Aspect ratio of cell body on each substrate (n = 50 for all substrates, $*p < 0.05$, $**p < 0.005$). Scale bars represent 20 µm.

Nuclear deformation coincides with cell elongation

To assess whether the ECM mechanical signals can be transmitted to the nucleus, we stained nucleus with Hoechst dye following the cell stretching and analyzed the shape of nuclei on different substrate. We found that nuclear deformation coincides with cell elongation in MDCK cells (Fig. 2). This effect is also modulated by substrate stiffness. On softer substrate, cells consistently show larger elongation and larger nucleus deformation. However, on soft substrate the thick stress fibers were not observed, therefore, mechanical stress arising from tension in stress fibers are not responsible for the nuclear deformation. We have previously reported that nuclear deformation in MDCK cell under shear stress is mediated by intracellular Ca^{2+} that increases through activation of Piezo1 channels [8].

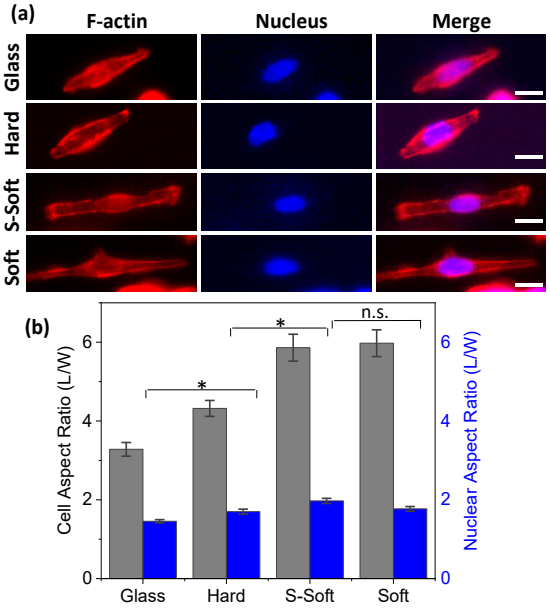

Figure 2. Nuclear deformation coincides with cell elongation. Images of actin (red) and nuclei (blue) in cells grown on micro-stripes on different substrates, showing soft substrate caused larger cell and nuclear deformation. Scale bar = 20 µm. (b) Statistical analysis of cell elongation and corresponding nuclear deformation (n = 40 for hard and semi-soft substrates; n = 30 for glass and soft substrates. $*p < 0.005$).

Role of Piezo1 in cell response to substrate confinements

MDCK cells endogenously express mechanosensitive Piezo1 channels. To investigate the role of Piezo1 in cell elongation, we targeted Piezo1 miRNA to knockdown Piezo1(P1KD). Cells were transfected with Piezo1 miRNA and co-expressed with EGFP to verify transfection (Fig. 3a). Knockdown of Piezo1 significantly reduced the elongation on patterns on glass substrate (Fig. 3b). The mean elongation of P1KD cells was ~ 2.5, that was 40% lower than nearby control cells. The statistical analysis shows consistent results (Fig. 3c). This result shows that Piezo1 mediated Ca^{2+} uptake participated in the cell elongation on hard substrate, which is consistent with our previous findings [3]. Our recent results show that inhibiting Piezo1 only reduced the stress fibers, however, it did not alter the cell shape (data not shown). It suggests that different stiffness may activate different mechanisms for MDCK cell elongation.

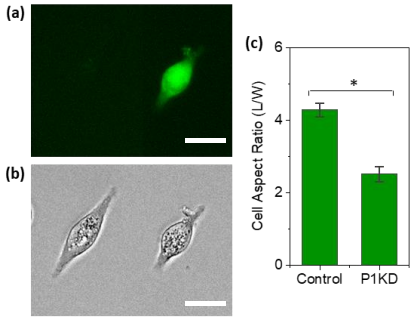

Figure 3: Elongation of Piezo1 knockdown cells. (a) MDCK cells expressing miRNA that target *Piezo1* (green). **(b)** Elongation of control cell (left) and P1KD cell (right), showing that Piezo1 knockdown significantly reduced elongation. Scale bar = 20 µm. **(c)** Mean cell elongation in P1KD and control, showing Peizo1 knockdown reduced elongation by 40% (Control: n = 29; P1KD: n = 14, $*p < 0.005$).

DISCUSSION

MDCK cells elongate on micropatterned stripes, the cell response depends on the substrate stiffness. Cells showed maximal elongation on the soft substrate compared to stiffer ones. Nuclear deformation coincides with the cell elongation. It has been reported that mechanical confinements change tension in actin stress fibers, causing changes in cell shape and nuclear deformation [5, 7]. Our results show that the cytoskeleton tension may not be responsible for deformation, depending on the substrate stiffness. On stiffer substrates, Piezo1 mediated Ca^{2+} uptake may cause increase in myosin contractility and cytoskeletal tension resulting in cell expansion. On soft substrates, stress fibers were reduced significantly, cytoskeleton may only serve as guidelines for cell adhesion. Epithelial cells utilize different force transmission mechanisms in response to varying local stiffness.

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REFERENCES

[1] Suffoletto K, et al. J Biomech. 2014;48:627-35.
[2] Thery M, et al. Nat Cell Biol. 2005;7(10):947-53.
[3] Jetta D, et al. Sci Rep. 2021;11(1):5088.
[4] Thery M, et al. Cell Motil Cytoskeleton. 2006;63(6):341-55.
[5] Versaevel M, et al. Sci Rep. 2014;4:7362.
[6] Swift J, et al. Science. 2013;341(6149):1240104.
[7] Bray MA, et al. Biomaterials. 2010;31(19):5143-50.
[8] Jetta D, et al. J Cell Sci. 2019;132(11).