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Supporting Material

Cell shape and substrate rigidity both regulate cell stiffness.

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Materials and Methods

Cell lines and reagents

Bone marrow-derived human mesenchymal stem cells (Lonza) were cultured in DMEM (GIBCO) with 1 g/L D-glucose, 0.3mg/ml L-glutamine and 100 mg/L sodium pyruvate, 100 units/ml penicillin, 100 mg/ml streptomycin and 10% heat inactivated fetal bovine serum (GIBCO) and maintained at 37^{o}C and 5% CO_2. Early passages of cells (P3-P6) were used in experiments. Unless otherwise specified all reagents are analytical grade and purchased from Sigma.

Substrate and cell shape

Polyacrylamide gels of different stiffness were prepared with mixed acrylamide and bis-acrylamide solutions (Fisher, Hampton, NH) by polymerizing with N,N,N9,N9-tetramethylethylenediamine and ammonium persulfate as described previously (1, 2). The gel is then activated with sulfosuccinimidyl 6 (4-azido-2-nitrophenyl-amino) hexanoate (Sulfo-SANPAH; Pierce) and adhesion proteins were covalently ligated through free amino groups by succinimide chemistry. The gel surfaces are coated with 0.1 mg/ml salmon fibronectin (Sea Run Holdings, Freeport, ME). hMSCs are cultured on these gels for about 16 hours before conducting experiments.

To control substrate stiffness, hMSCs were grown on microfabricated polydimethylsiloxane (PDMS) post array detectors (mPADs) of diameter 1.8 um as previously described (3, 4). Briefly, mPADs were microcontact printed with a saturating concentration of 50 μg/ml fibronectin (BD Biosciences) so that cells cultured on the devices spread and conformed to the printed patterns. The cells were cultured on mPADs in the presence of growth medium for 16 hours before performing atomic force microscopy or immunostaining.

Atomic Force Microscopy

To measure stiffness, force indentation curves at cell cortex were obtained as previously described (5) on a Veeco Bioscope I using a silicon nitride probe with a cantilever spring constant of 0.06 N/m (DNP, Veeco). By fitting the first 400nm of these indentation curves to the Hertz model (6), the stiffnesses of cell cortex are obtained. Typical indentation regions are shown in white circles in Fig. S1. These regions are chosen to be far from the lamellipodia and nucleus and typically range in thickness from 3-10 um; these regions are even thicker for cells on smaller islands. Since these regions are thick compared to the indentation depths, substrate stiffness does not significantly influence cell stiffness measurements. As a check, a small surface map of cell stiffness is measured (Fig. S2). Since the underlying microposts are 2 MPa in stiffness and the centroid to centroid distance of these posts is 4 um, the substrate stiffness

effectively varies from 2 MPa to 0 Pa (no micropost) every 4 um. If the substrate influenced the AFM measurements, correlations in the stiffness map on the lengthscale of 4 um would be expected. However, no such correlations were detected. Since we measure at least 15 cells for each data point and average over 3 regions per cell, our final stiffness measurement is an ensemble average over both cortical actin and stress fibers if they are close enough to the dorsal cell surface.

Immunostaining

Cells were fixed with 4% para-formaldehyde (Electron Microscopy Science) in PBS, washed, incubated in 0.2% Triton buffer, followed by incubation with a vinculin primary antibody (Sigma Aldrich) and another incubation with fluorophore-conjugated secondary antibody. To detect actin cytoskeleton, a fluorophore-conjugated phalloidin is used.

Image Analysis

Samples were taken with an inverted micro- scope (Zeiss Axiovert 200M; Carl Zeiss MicroImaging) with a 40× (1.3 NA, oil immersion; EC Plan NEOFLUAR) objective. Images taken from a Peltier-cooled monochrome charge-coupled device (CCD) camera (AxioCam HRM) are then analyzed with ImageJ.

Traction forces of cells were obtained as previously described (7). Briefly, fluorescent images of the Δ9-DiI-stained PDMS microposts were acquired at two different focal planes; one at the top of the posts and one at 1 um above the base of the microposts. The centroids of the posts of both the top and bottom planes are determined by a thresholding algorithm. By quantifying the difference between the top plane of the posts (which would be deflected by traction forces) and the bottom plane of the posts (un-deflected), the deflection caused by cells is obtained. The traction forces are computed by multiplying the deflection by the micropost spring constant obtained by FEM simulation.

References

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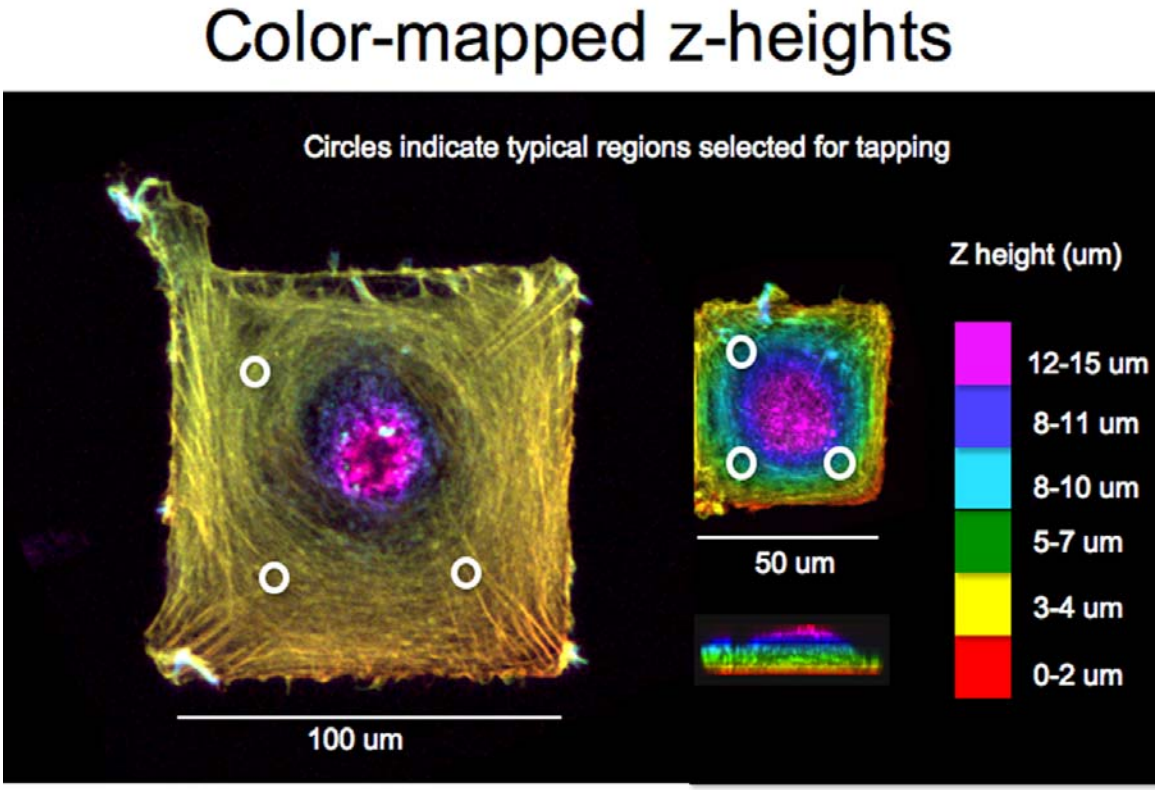
Online supplemental materials:

Figure S1. Z-height of hMSC plated on microposts. hMSC were plated on fibronectin adhesive islands, fixed and stained with fluorophore-conjugated phalloidin. Confocal stacks are used to reconstruct z-height of the cells. (A) On a 100 x 100 um island, hMSC is mostly flat as seen the mostly yellow color map (3-4um) but there are small edges of lamellipodia regions (red) too; the nucleus is about 12-15 um tall (pink) (B) When plated on a smaller 50 x 50 um island, hMSC assume a more three dimensional shape as indicated from the z-color map. White circles indicate typical regions of the cells chosen for AFM tapping. These regions typically range from 3-10 um in cell height and are far from both the nucleus and lamellipodia.

Figure S2. Stiffness map of a region of cortex of a hMSC plated on microposts of 18 nN/um on a 50 x 50 um island. The stiffness map shows variations in stiffness that fluctuate around 4000 Pa but no stiffness correlations corresponding to the 4 um centroid to centroid distance of microposts. The average stiffness is 4006 Pa with a standard deviation of 892 Pa.

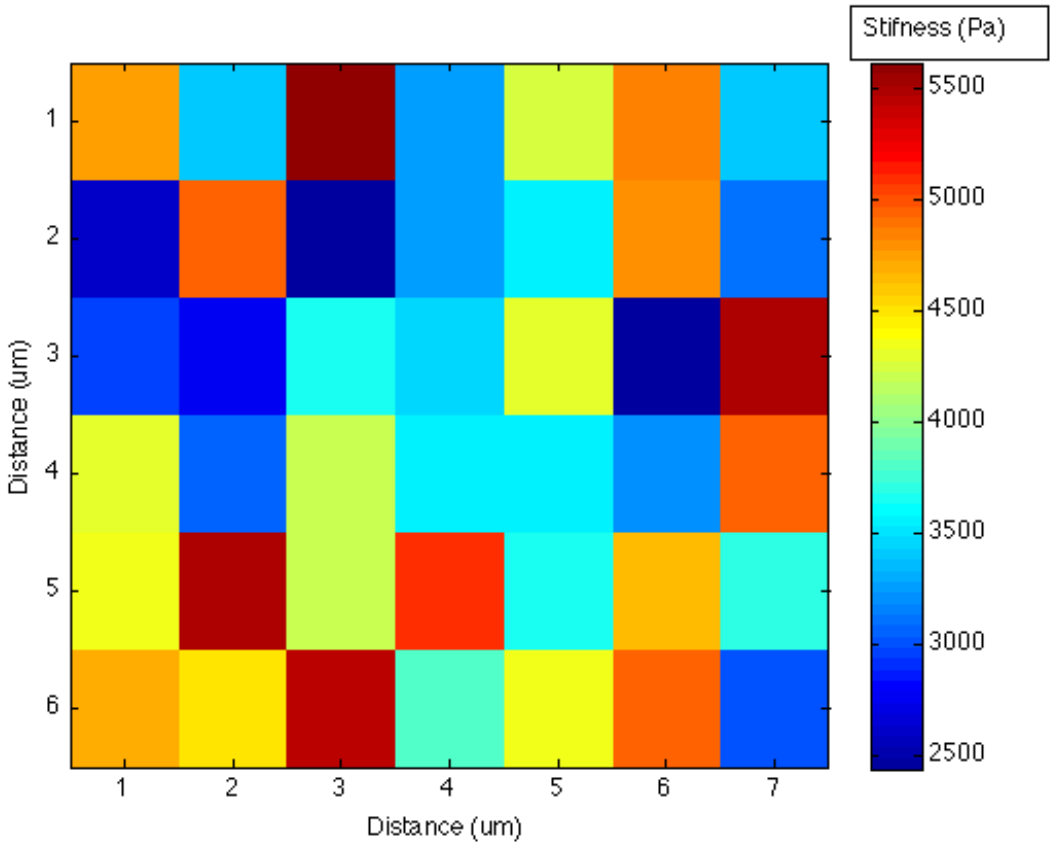
Figures

Fig. S1



hMSC on stamped microposts stained with phalloidin

Fig. S2


Stifness (Pa)
5500
5000
4500
4000
3500
3000
2500
Distance (um)
1
2
3
4
5
6
7
Distance (um)
1
2
3
4
5
6