## Supplementary Note 1: Experimental Methods

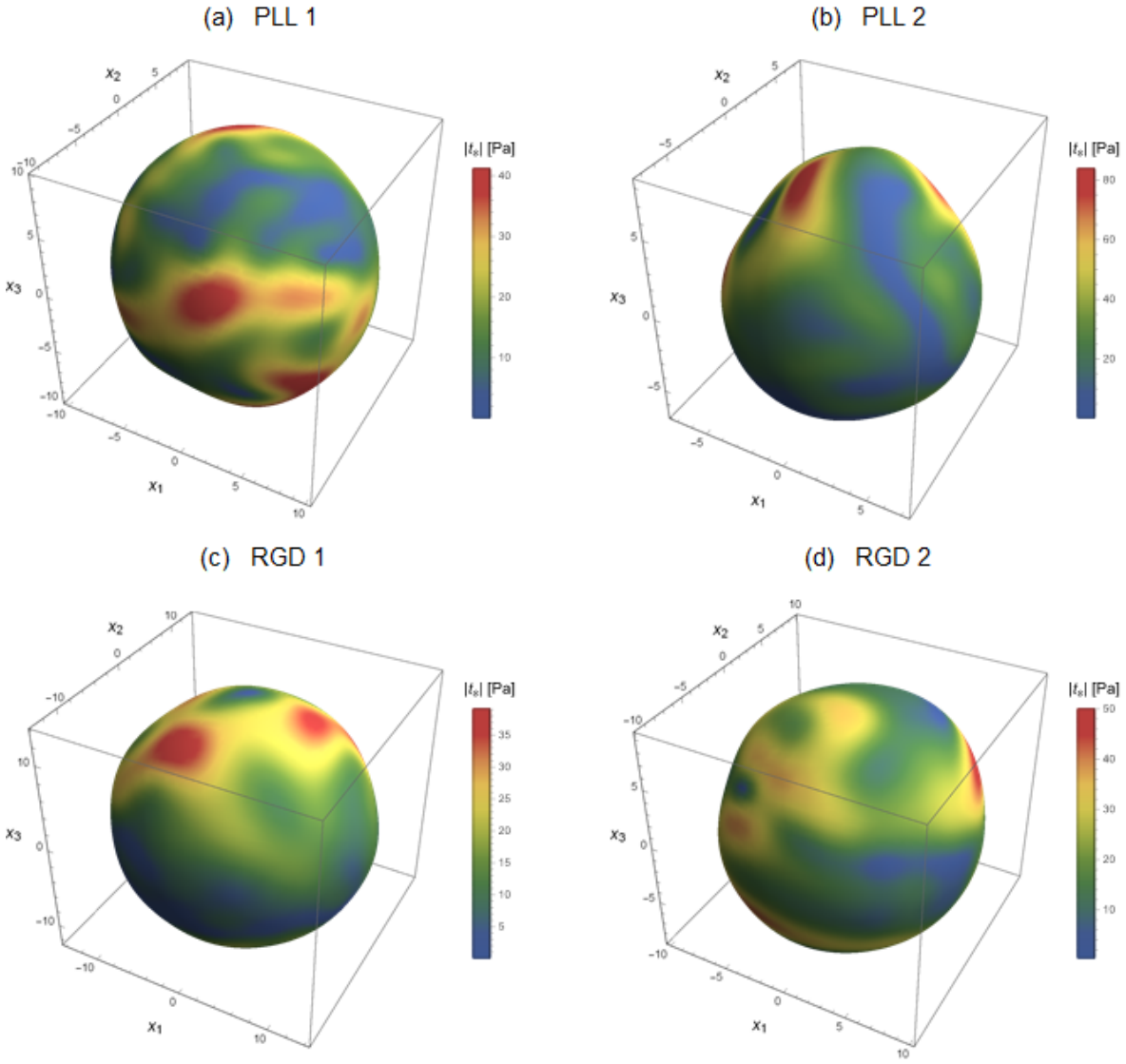
**Microsphere synthesis.** To synthesize hydrogel microspheres, a "pregel-in-oil" microemulsion was created, containing an aqueous gel pre-cursor phase stabilized by 9.1 mM Span 80 and 0.9 mM Tween 20, common non-ionic surfactants, in a continuous food-grade oil phase. 50 µL of gel pre-cursor was prepared by mixing poly(ethylene glycol) diacrylate monomer with $M_n$=700 g/mol (PEGDA-700; Sigma), 1.1 mM lithium phenyl (2,4,6-trimethylbenzoyl) phosphinate photoinitiator (Tokyo Chemical Industry), 0.4 mg/mL rhodamine-acrylate (Polysciences Inc.), and 2 mM biotin-PEG-acrylate (Creative PEGWorks) and was emulsified in the oil-surfactant solution under strong vortexing for several seconds. Photopolymerization for 1 hour in a UV curing chamber [KGW-94N; KAIS] of the microemulsion resulted in the formation of chemically-crosslinked microspheres functionalized with biotin and labelled with rhodamine. After curing, the microspheres were rinsed several times with deionized water via centrifugation and resuspension to remove oil and unreacted materials. The resulting microspheres were functionalized with Neutravidin by centrifugation and resuspending in 1 mL of 1 µM Neutravidin (ThermoFisher). The Neutravidin-coated microspheres were then rinsed of excess Neutravidin, resuspended in a 6 mM solution of the desired biotinylated adhesion molecule and subsequently stored in cell culture media until used within 2 days.

**Bulk modulus measurements using microspheres.** Microspheres were synthesized as described and coated with a biotinylated poly-L-lysine (Alamanda Polymers) and equilibrated with 1X phosphate buffered saline (PBS). Dilute solutions of microspheres were seeded into custom-made cover-glass bottomed wells and allowed to adhere for 3 hours before vigorous rinsing to remove weakly attached microspheres. An osmotic stress was externally applied by the addition of a solution containing fluorescein-labeled, high molecular-weight (HMW) dextran ($M_w$=500 kD) (Sigma; FD500). Volumetric images of microspheres were obtained using a Leica SP8 confocal microscope and volume-change $\Delta V$ was determined using ImageJ by measuring the change in microsphere radius $R$ from the difference in maximum-intensity projections before and after solution exchange: $\Delta V = \left|\frac{4}{3}\pi\left(R_{after}^3 - R_{before}^3\right)\right|$. In practice, microspheres were first imaged in PBS to obtain the unloaded volume. To apply osmotic compressive stresses, PBS was removed, and solutions of dextran were added and allowed to equilibrate for 5 minutes, after which time no change in microsphere volume was observed. To increase the osmotic compressive stress, dextran solutions were removed and samples were rinsed several times with PBS and allowed to equilibrate before the next dextran solution was added. The bulk modulus was determined from a linear fit of the data, which is plotted as applied osmotic pressure versus average volumetric compressive strain $\Delta V/V_0$, where $V_0$ is the initial sphere volume in the absence of dextran (as shown in **Fig. 1e**; $n$=15, 3 experiments). The uncertainty in $\Delta V/V_0$ (given by error bars in **Fig. 1e**) is estimated as the standard deviation from measurements. The fitting procedure was performed in IgorPro using a chi-squared minimization approach via the Levenberg-Marquardt algorithm to determine the best-fit slope of the line, with the $y$-intercept fixed to 0 and with weighting given by the standard deviation.

**Cell culture.** Mouse fibroblast (NIH 3T3s) and were obtained from the American Type Culture Collection (ATCC) and maintained in high glucose Dulbecco's Modified Eagle's medium (DMEM) supplemented with 10% calf bovine serum and 1% penicillin-streptomycin (Life Technologies). Cells were grown in 25 $cm^2$ rectangular canted-neck cell culture flasks with vent-caps (Corning Life Sciences) at 37° C and 5% carbon dioxide, and were either passaged or used for experiments at 60-80% confluency. For experiments, cells were stained for nuclei using Hoechst-3342 (Life Technologies) and F-actin using a SiR-actin kit (Cytoskeleton Inc.).

**Formation of fibroblast aggregates.** To form fibroblast aggregates, PEGDA microspheres functionalized with 10 μM of either biotinylated-poly-L-lysine hydrobromide (Alamanda Polymers) or biotinylated-RGD peptide (Peptides International) were added to solutions of suspended fibroblasts at a 1:30 ratio (microspheres:cells) and immediately seeded into poly-L-lysine (0.1%; Sigma) treated custom glass-bottomed coverslip microwells at a density of 150,000-200,000 cells/well. Fibroblasts were allowed to form attachments and deform microspheres overnight at 37°C and 5% carbon dioxide. Cells and deformed microspheres were then transferred to our microscope facility and imaged as described below.

**Imaging of microspheres in cell aggregates.** Imaging was performed using an inverted Leica TCS-SP8 spectral confocal microscope with a 40X water-immersion objective (numerical aperture, NA = 1.1), at a 1000 Hz acquisition rate, with a pinhole diameter of 1 AU. Samples were placed in a humidified environmental chamber maintained at 37 ºC and 5% $CO_2$. In practice, we first identified several deformed microspheres by eye and saved their locations to allow for subsequent high-resolution imaging of these regions of interest. To minimize fluorescent bleed-through between imaging channels, line-sequential scans were taken. The first scan used 405-nm excitation with a 430-nm to 500-nm detection window to image the Hoechst-stained nuclei. The second scan used 555-nm excitation with a 600-nm to 680-nm detection window to image the diffuse, volumetric rhodamine-B labeling of the microspheres. The third and final scan used a 652-nm excitation with 665-nm to 730-nm detection window to image the stained filamentous actin. For each location, a field of view with typical in-plane area of 50-100 $\mu m^2$ and 20-75 μm stack height (along the optical axis *z*) was imaged; each scan took approximately 1-2 minutes to collect depending on the *z*-height. This resulted in a stack of 2D images with typical *x*-*y* magnification of 94.7 nm/pixel with a separation distance of ~400 nm between image planes in *z*. The pixel residence time was typically 225 ns, resulting in a collection time of 23.5 s per excitation wavelength for a typical sphere of ~ 20 μm diameter. After imaging the deformed configurations, all cells were lysed for several hours using a low concentration sodium dodecyl sulfate solution (<0.1% w/v) (Sigma). After lysis was complete and the actively-generated cellular loading removed, the load-free microspheres were imaged again, as described. Images were converted to multipage TIFF format, and microspheres were identified and manually cropped to isolate a single object of interest.




| (e) | | PLL 1 | PLL 2 | RGD 1 | RGD 2 |
|---|---|---|---|---|---|
| $\langle t_s \rangle$ | (Pa) | 17.7 | 30.1 | 13.4 | 21.2 |
| $\lvert t_s \rvert^{min}$ | (Pa) | 0.15 | 0.3 | 0.1 | 0.38 |
| $\lvert t_s \rvert^{max}$ | (Pa) | 41.2 | 83.8 | 39 | 50 |
| SD | (Pa) | 8.5 | 16.2 | 8.3 | 9.6 |

**Figure S1:** Shear tractions for **a,b** PLL-coated and **c,d** RGD-coated microspheres, respectively, embedded in 3T3 fibroblast aggregates. **e,f** Table of mean traction force values, as well as the extremal traction magnitudes and the standard deviations for each sensor shown.

**Movie S1**: Animation showing 3D normal traction map for RGD-coated microsphere (RGD 1)

**Movie S2**: Animation showing 3D shear traction map for RGD-coated microsphere (RGD 1)

**Movie S3**: Animation showing 3D normal traction map for PLL-coated microsphere (PLL 1)

**Movie S4**: Animation showing 3D shear traction map for PLL-coated microsphere (PLL 1)

**Movie S5**: Animation showing 3D normal traction map for RGD-coated microsphere (RGD 2)

**Movie S6**: Animation showing 3D shear traction map for RGD-coated microsphere (RGD 2)

**Movie S7**: Animation showing 3D normal traction map for PLL-coated microsphere (PLL 2)

**Movie S8**: Animation showing 3D shear traction map for PLL-coated microsphere (PLL 2).