

Electronic Supplementary Information

Detailed methods for AFM characterization

We used atomic force microscopy (alpha300A, WITec, Knoxville, TN) to investigate the elastic moduli of assembled gels. A 10 μm radius glass bead was glued to the tip of the cantilever (PPP-NCSTAuD, $k = 7.2 \text{ N/m}$, Nanosensors, Neuchatel, Switzerland) prior to indentation.¹ Polyacrylamide IPNs were polymerized similar to procedure described above; however, we used 45 μL of solution with a 25 mm coverslip, resulting in gels $\sim 150 \mu\text{m}$ thick. Gels equilibrated in PBS overnight at 25 $^{\circ}\text{C}$ before indentation testing. Indentation results (10 $\mu\text{m/s}$ indentation rate) are fit to the Oliver-Pharr model^{2,3} to yield elastic modulus.

Detailed methods for strain transfer characterization of composite substrates

To characterize the macroscale behavior of the substrates, we used ImageJ (National Institutes of Health) to measure the height h and width w of the silicone in initial (“null”) and stretched (“strain”) states (Fig. S1). We then calculated the engineering strain, e.g.

$$(h_{\text{strain}} - h_{\text{null}}) / h_{\text{null}}$$

Also in ImageJ, an ellipse was drawn around the gel, and the major axis and minor axis were determined using the “Measure” function to calculate the engineering strain for the gel surface.

To confirm microscale strain was similar to observed macroscale strain, fiducial markers visible in bright field and fluorescence were placed on the surface of the silicone and gels, respectively (Fig. S2). Dark dots visible in bright field images were made by applying a silicone formulation containing carbon black (Sylgard 170, Part A, Dow Corning) to the surface of silicone strips with a cotton swab before polymerizing IPNs in place. The cover slips used during formation of IPNs had fluorescent beads adsorbed onto the surface (1 μm diameter, Thermo Fisher Scientific, Waltham, MA), thus transferring fiducial markers visible by fluorescence imaging to the surface of the gel.

Images were captured in both bright field (focal plane of the silicone surface) and fluorescence (focal plane of the beads at the gel surface) in unstrained and strained conditions (DMRXA2 upright microscope, Leica Microsystems, Bannockburn, IL with Orca R2, Hamamatsu, Hamamatsu City, Japan). For “microscale” calibration, the sample was strained approximately 10%. The displacement of markers in each image set were analyzed using custom MATLAB code based on image correlation algorithms implemented in PIVlab (www.pivlab.blogspot.com). Based on the resulting displacement vectors, we evaluated the Lagrangian strain tensor for each element defined by the regularly spaced 50 μm grid. To minimize the effect of erroneous boundary calculations (obvious in Fig. S2B, for example), we selected a consistent 500 μm x 500 μm area of the image and recorded the trimmed mean (average of the middle 50% of values) for this area. Over 75% of the acquired paired- paired images (unstrained and strained for both silicone and gel surfaces) could be processed in this manner.

To assess strain transfer from the gel to the cell monolayer, images of the beads with red fluorescent filters and the cells using differential interference contrast filters were taken at null condition and then took pictures at the strain condition. PIVlab was used again on the paired-paired images, and the engineering strain was calculated from these data (Fig. S2).

Cell culture methods

To bond proteins to polyacrylamide gels, we pooled 1 μM N- sulfosuccinimidyl-6-(4'-azido-2'-nitrophenylamino) hexanoate (sulfoSANPAH, G Biosciences, St. Louis, MO) suspended in PBS onto the gels and treated with ultraviolet light (10.8 J/cm^2 , $\lambda_{\text{UV}} = 383 \text{ nm}$). We then rinsed the gels with PBS and repeated the sulfoSANPAH treatment. PBS solution (100 μl) containing either 172 $\mu\text{g}/\text{ml}$ fibronectin (Human plasma, BD Biosciences, San Jose, CA) or 172 $\mu\text{g}/\text{ml}$ laminin (Ultrapure mouse, BD Biosciences) was pooled on the gels for 2 hours. Between two and four composite substrates were arranged in a 100 mm petri dish. Human dermal microvascular ECs⁴ (courtesy of the Cooke Lab, Stanford) or human aortic SMCs (Cell Systems, Kirkland, WA) at a density of ~ 3 million cells/ml were then plated on fibronectin- or laminin-coated gels and allowed to attach for 30 mins. Media (10 ml) was added to remove unattached cells, and the substrates were incubated at 37 $^{\circ}\text{C}$ overnight. Only regions of $>80\%$ confluence were included for analysis.

References

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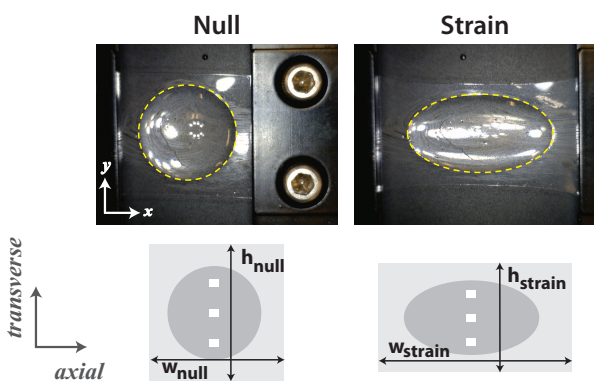


Figure S1. Images and schematic of macroscale calibration process; gels are 12 mm across in *null* condition. Width, w , and height, h , are used to calculate engineering strain of the silicone and axes of ellipses fit to edge of gels (yellow dashed lines) are used to calculate engineering strain of the gel. White boxes indicate regions imaged for microscale calibration.

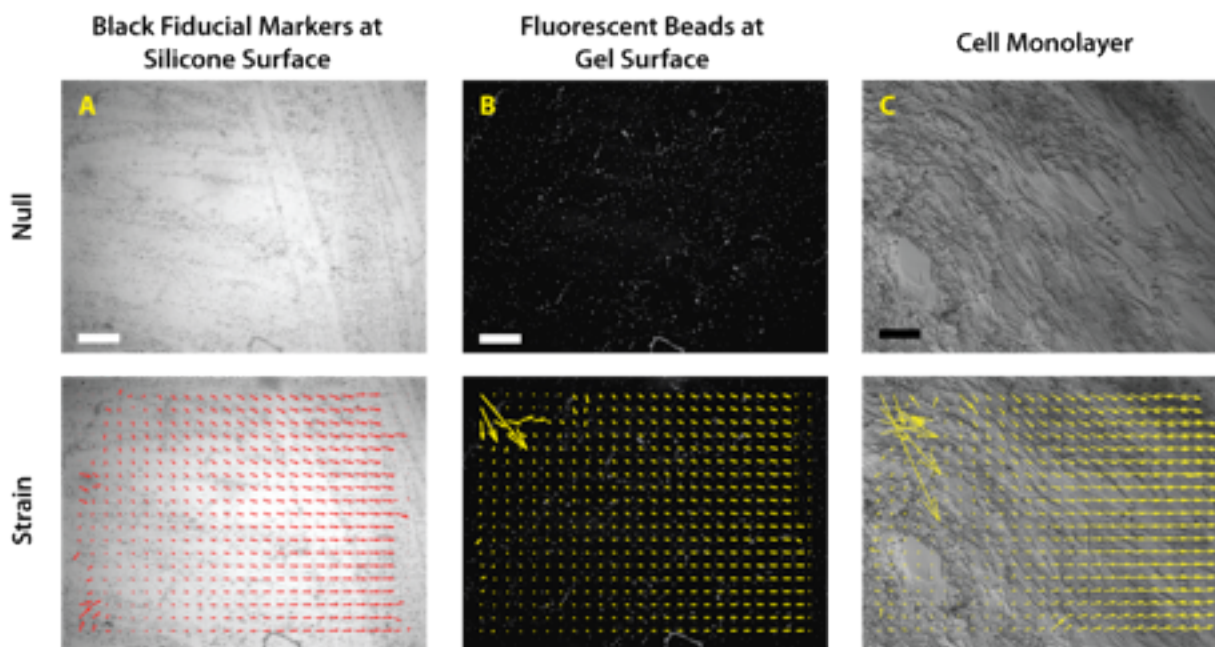


Figure S2. Images and representative analysis for microscale calibration pictures depicts displacement vectors calculated for image pair characterizing the (B) HT-6240 silicone surface, (C) 20 kPa gel surface, and (D) SMCs attached to fibronectin-coated 20 kPa gel. Inconsistent vectors along the outer perimeter of the image were disregarded during strain calculations by using vectors in a consistent 0.25 mm² region. Scale bar = 100 μ m.