

Traction Force Screening Enabled by Compliant PDMS Elastomers

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ABSTRACT Actomyosin contractility is an essential element of many aspects of cellular biology and manifests as traction forces that cells exert on their surroundings. The central role of these forces makes them a novel principal therapeutic target in diverse diseases. This requires accurate and higher-capacity measurements of traction forces; however, existing methods are largely low throughput, limiting their utility in broader applications. To address this need, we employ Fourier-transform traction force microscopy in a parallelized 96-well format, which we refer to as contractile force screening. Critically, rather than the frequently employed hydrogel polyacrylamide, we fabricate these plates using polydimethylsiloxane rubber. Key to this approach is that the polydimethylsiloxane used is very compliant, with a lower-bound Young's modulus of ~ 0.4 kPa. We subdivide these monolithic substrates spatially into biochemically independent wells, creating a uniform multiwell platform for traction force screening. We demonstrate the utility and versatility of this platform by quantifying the compound and dose-dependent contractility responses of human airway smooth muscle cells and retinal pigment epithelial cells. By directly quantifying the endpoint of therapeutic intent, airway-smooth-muscle contractile force, this approach fills an important methodological void in current screening approaches for bronchodilator drug discovery, and, more generally, in measuring contractile response for a broad range of cell types and pathologies.

INTRODUCTION

Many adherent cells employ actomyosin contractility to exert traction forces on their surroundings. These forces are an essential part of cellular deformation (1–3), adhesion (4–6), spreading (7), and migration (8–10), as well as growth (11), homeostasis (12,13), gene expression (14), and apoptosis (15). The significant role of traction force makes it a novel principal therapeutic target in diverse diseases; however, accurate measurements of traction forces are essential for this approach.

To quantify cell traction forces, researchers have employed a variety of techniques and tools. From the first wrinkling thin silicone sheets (16) to complex three-dimensional multicellular contractility (17), a multitude of biomechanical methods have been developed, collectively referred to

as traction force microscopy (TFM), as reviewed here (18). Although these approaches have enabled the discovery of valuable mechanobiological connections, these methods are generally inherently slow and restricted to low-throughput implementation, limiting their utility as tools in broader pharmacological applications.

To address this need, we employ Fourier-transform TFM in a parallelized 96-well format, an approach we refer to as contractile force screening (CFS). Critically, rather than using the frequently employed hydrogel polyacrylamide (PAA), we fabricate these plates using polydimethylsiloxane (PDMS) rubber. Key to this approach is that the PDMS used is very compliant, with a lower-bound Young's modulus of ~ 0.4 kPa, unlike commonly used Sylgard 184 formulations (additional detailed mechanical characterization is presented in the [Supporting Materials and Methods](#)). Like PAA, soft PDMS elastomers possess several material-favorable properties: their stiffness is tunable over a large physiological range ([Fig. 1](#)), and they are nontoxic, nondegrading, and biologically inert. In addition to these aspects, this compliant PDMS has numerous advantages over

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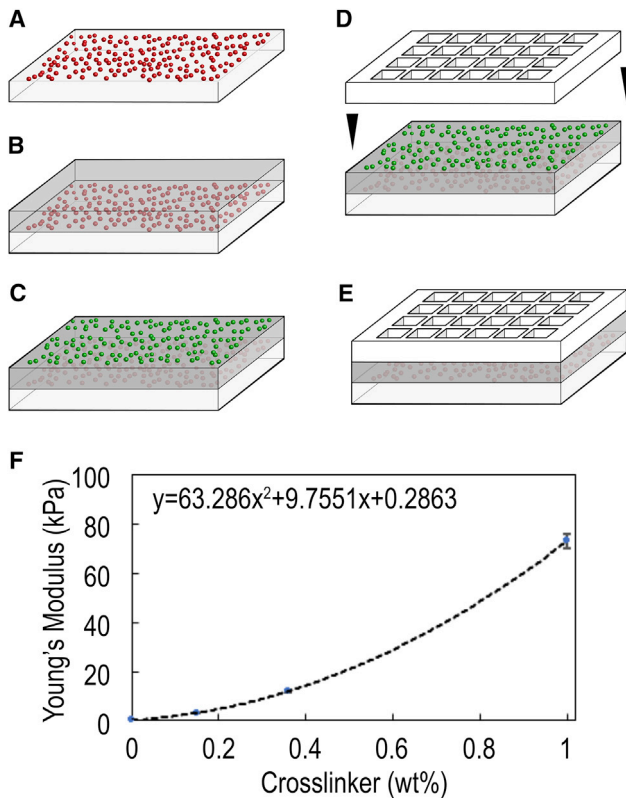


FIGURE 1 Fabrication of multiwell substrates and mechanical characterization of tunable PDMS elastomer. Multiwell PDMS devices for TFM are fabricated by (A) optionally coating a layer of fluorescent microspheres (for de-drifting images) on the custom glass slide, (B) spin-coating a $\sim 100\text{-}\mu\text{m}$ -thick layer of compliant PDMS, (C) spin-coating a $\sim 1\text{-}\mu\text{m}$ -thick layer of compliant PDMS mixed with custom-synthesized beads with different fluorescence emission for measuring cell-induced deformation of the PDMS, (D) bonding a multiwell partitioning strip to the top, and (E) ligating and culturing cells. (F) A graph of PDMS moduli as determined by shear rheology ($n = 4$ independent preparations per data point) is shown. (G) A table of same-mean PDMS moduli \pm SD is shown. To see this figure in color, go online.

PAA: 1) it is optically transparent, with a refractive index of ~ 1.4 , which is comparable to glass; 2) it is indefinitely stable after production without special storage considerations; 3) it is amenable to spin coating, providing a simple means of creating a uniform and flat surface; and 4) it is a uniformly nonporous surface, unlike PAA, whose porosity can vary strongly with cross-linking concentration (19). Critically, the monolithic, impermeable nature of these silicone substrates makes them easy and ideal to subdivide spatially into biochemically independent wells, creating a uniform multiwell platform for TFM. Taken together,

our compliant PDMS presents numerous advantages in becoming a new standard in soft substrates for TFM; these advantages are particularly important for a standardized higher-throughput technology and will enable widespread adoption of CFS from our previous approach using PAA (20).

METHODS

Cell culture

Primary human airway smooth muscle (ASM) cells were obtained from the Gift of Hope Organ and Tissue Donor Network (Itasca, IL). These cells have been well-characterized previously, e.g., (21). All measurements were performed using cells at passage 5–8 from two nonasthmatic donors. ARPE-19 (retinal pigment epithelium) cells were obtained from American Type Culture Collection (Manassas, VA). All culture media formulations are provided in the [Supporting Materials and Methods](#).

Preparation of silicone substrates in custom 96-well plates

We fabricate our multiwell TFM dishes by applying very compliant and tunable modulus PDMS onto custom-cut glass slides and then partitioning the wells with a plastic subdivider (Fig. 1 A–E). In brief, very compliant commercial PDMS (NuSil 8100; NuSil Silicone Technologies, Carpinteria, CA) is mixed with a small percentage by weight of Sylgard 184 cross-linking agent to make a tunable ($E = 0.4\text{--}73$ kPa) substrate, which is impregnated with an $\sim 1\text{-}\mu\text{m}$ -thick layer of fiduciary particles to reveal cell-induced deformations. This approach differs from existing PDMS TFM strategies, as this substrate is comparably compliant to PAA and linearly elastic, yet not a hydrogel. Full details of plate preparation, including detailed substrate functionalization and mechanical characterization, are provided in the [Supporting Materials and Methods](#).

Mechanical characterization of PDMS substrates

We measured the frequency-dependent storage and loss-shear moduli for PDMS with different additional cross-linker formulations using shear rheology (MCR 302, 25-mm parallel plate tool; Anton Paar, Graz, Austria). Samples of ~ 0.6 mL were loaded and cured at 100°C for 2–3 h, the normal force was reset, and the shear modulus was measured at 1 Hz and 0.5% strain. Young's moduli, E , were calculated from shear moduli, G , as $E = 2 \times G(1 + \nu)$ by assuming the PDMS is incompressible with a Poisson ratio, ν , of 0.5 (Fig. 1 F and G). Further mechanical characterization is described in the [Supporting Materials and Methods](#).

Measurements of cell traction forces

The 96-well plate was mounted within a heated chamber (37°C) upon an automated computer-controlled motorized stage and imaged at $10\times$ magnification using a monochrome camera (DFC365 FX; Leica, Wetzlar, Germany) affixed to an inverted microscope (DMI 6000B; Leica). We acquired fluorescent images of microspheres embedded in the elastic substrate immediately underneath the cells at 1) baseline with no treatment, 2) after treatment, and 3) after cell detachment with trypsin (*reference null-force image*). By comparing the fluorescent images at reference with the corresponding images at baseline and after treatment, we obtain a time series of bead displacement and hence substrate deformation fields (resolution = ~ 15 μm). Using the measured substrate deformation, the pre-defined substrate modulus, and thickness, traction force maps and the root mean-squared value were calculated over a $732\ \mu\text{m} \times 732\ \mu\text{m}$ area on a

well-by-well basis, using the approach of Fourier-transform traction cytometry (22) modified to the case of cell monolayers (23).

Drugs

Histamine, isoproterenol, salbutamol, salmeterol, formoterol, thrombin, and H₂O₂ were purchased from Sigma-Aldrich (St. Louis, MO). Y27632 was purchased from EMD Millipore (Burlington, MA). Human VEGF-A¹⁶⁵ and bevacizumab were purchased from R&D systems (Minneapolis, MN) and Genentech (South San Francisco, CA), respectively.

Statistics

Statistical comparisons for traction differences were performed using the nonparametric Wilcoxon matched-pairs signed rank test. Differences were considered significant when $p < 0.05$.

RESULTS AND DISCUSSION

CFS entails quantifying the cell-generated forces by measuring fluorescent bead positions in each well of the 96-well plate: 1) without cells, 2) with cells adhered at baseline contractility, and 3) after treatment with the compound(s) of interest. For example, for a representative well of a 96-well plate (Fig. 2 A), shown are ASM traction force maps and the root mean-squared value (*inset*) at baseline (Fig. 2 D, 93 Pa), after treatment with the contractant, the H1 agonist histamine (Fig. 2 E, 112 Pa), and after additional treatment with the relaxant, the β 2 adrenergic receptor agonist isoproterenol (Fig. 2 F, 52 Pa).

First, we tested the suitability of our approach for higher-capacity measurements. We evaluated common factors associated with ASM contraction, including constituents of the culture medium and properties of the cellular substrate. Although serum deprivation only marginally affected the scope of ASM relaxation (contraction with 10 μ M histamine—relaxation with additional 1 μ M isoproterenol), substrate stiffness had a profound impact, with an optimal response on 12-kPa stiff substrates. Given these findings, we focused subsequent studies on 12-kPa stiff substrates prepared in 96-well plates. Individual wells of a representative plate were either assigned to a positive or negative control group. In the positive control group, cells were prestimulated with 10 μ M histamine to induce maximal contractility, followed by poststimulation with the relaxant, 10 μ M Y27632, for 30 min. In the negative control group, cells were prestimulated with vehicle (phosphate-buffered saline), followed by poststimulation with vehicle (phosphate-buffered saline) for 30 min. In both groups, traction-force-measured poststimulation was normalized to the corresponding prestimulation value on a well-by-well basis. From these measurements of normalized changes, we determined that the groups were statistically different ($p < 0.05$), as ascertained by an unpaired Student's *t*-test.

Next, we verified the utility of our approach in pharmacology by examining traction force changes induced by a

diverse set of well-known and clinically relevant ASM relaxation compounds (24). Each compound was evaluated in a 10-point dose-response manner across adjacent rows of the 96-well plate. Data were pooled from multiple plates and reported as a percentage of histamine response. The extent of ASM relaxation confirmed the known differences in potency of the β 2 adrenergic receptor agonists (salmeterol > formoterol > salbutamol > isoproterenol) (24), and the full agonist, formoterol, provided a greater scope of relaxation than the partial agonist, salmeterol, as expected (25) (Fig. 2 H; Table S1). Notably, as supported by negligible standard errors and the small coefficients of variation, the data were highly reproducible.

Here we have focused on ASM response; yet this approach is applicable in pharmacology to any adherent contractile cell type and is therefore expected to be of broad utility. In ASM, this need is particularly exigent, as current efforts to screen new ASM relaxation drugs employ indirect assay methods that are poorly predictive of functional response. Commonplace examples include the dissociation of intracellular calcium regulation from the effects of bradykinin, bitter tastants (26), and proton-sensing receptor ligands (27) on ASM contraction, a similar dissociation of cAMP regulation from bronchorelaxant effect (procontractile receptor antagonists and again bitter tastants), and the limited predictive utility of membrane potential for almost all drugs whether they target receptors or other contractile effectors or signaling elements. A more relevant screen that directly quantifies the target output of ASM relaxation, as does CFS, is required to efficiently test the pending generations of ASM relaxation drugs. To this end, CFS fills an important methodological void in ASM biology, and, more generally, in measuring contractile response for a broad range of cell types and pathologies.

To demonstrate the versatility of CFS, we examined a key pathogenic mechanism common to many ocular pathologies—dysfunction of the retinal pigmented epithelium (RPE) (28). We discovered that the RPE barrier-disruptive agent thrombin (29), the proangiogenic cytokine VEGF-A (30), and the oxidative stressor H₂O₂ (31) each caused an increase in RPE traction forces (Fig. 3). Conversely, the rho kinase inhibitor Y27632 or the VEGF-A inhibitor bevacizumab ablated these forces. Taken together, these data reveal a novel role for traction force increase in RPE dysfunction and advocate for new discovery efforts targeted at reducing these forces. This might be especially pertinent to offset RPE dysfunction in the commonly occurring dry form of macular degeneration (32), wherein no therapeutic intervention currently exists.

CONCLUSIONS

We have demonstrated utility for a 96-well silicone-based substrate for CFS. This approach is advantageous over CFS using PAA (20), as the material itself is more robust

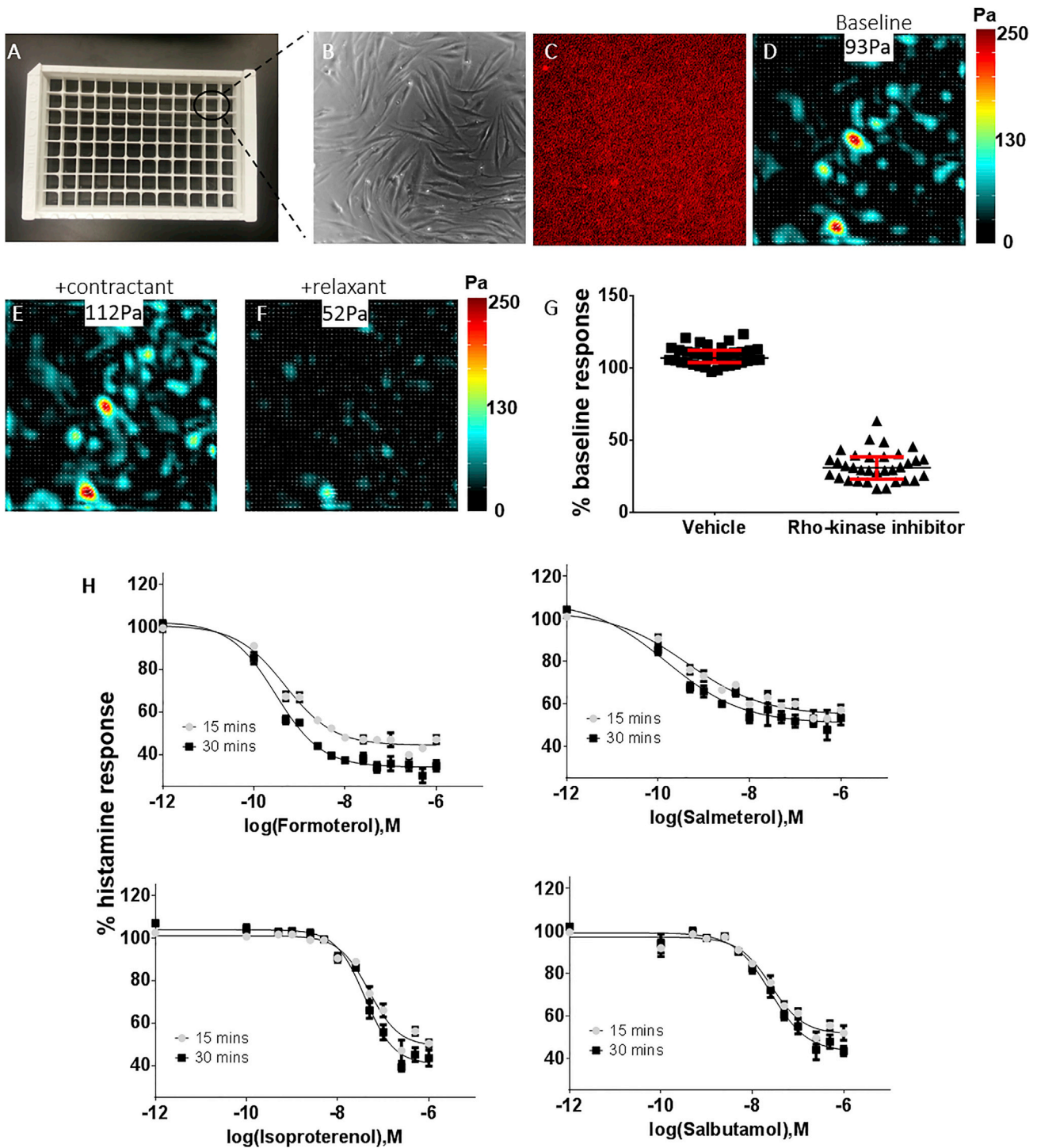


FIGURE 2 CFS using soft elastomeric substrates recapitulates known ASM pharmacological responses. Human ASM cells were cultured to confluence upon Young's modulus = 12 kPa (0.36% cross-linker) collagen-coated 96-well silicone substrates. (A–D) For a representative well of a 96-well plate, images of cells, fluorescent beads, traction force maps, and average magnitude (*inset*) at baseline are shown. (E and F) For the same well, shown are traction force maps and the average magnitude (*inset*) with the contractant compound histamine (10 μ M, 30 min), and after additional treatment with the relaxant compound isoproterenol (0.5 μ M, 30 min). (G) Over the 96-well plate, the force measurements are statistically different ($p < 0.05$) between positive and negative controls, as ascertained by an unpaired *t*-test. (H) Force measurements confirmed known differences in potency among a panel of functionally diverse ASM relaxation compounds (formoterol > salmeterol > salbutamol > isoproterenol). Plotted are the mean \pm standard error calculated from three to eight wells per dose per ASM relaxation compound. Data were pooled from two to four 96-well plates tested on different days but under identical experimental conditions. To see this figure in color, go online.

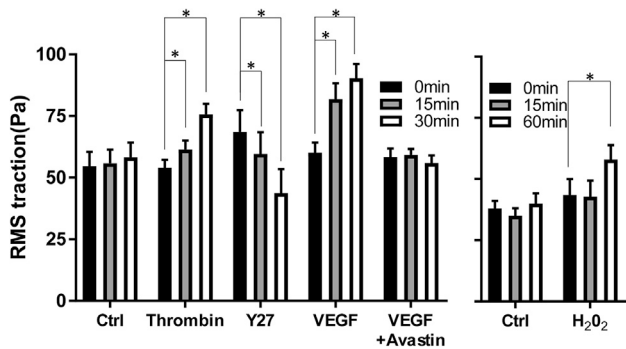


FIGURE 3 Mediators of retinal epithelial dysfunction increase cell traction forces. Human ARPE-19 cells were cultured to confluence upon Young's modulus = 12 kPa (0.36% cross-linker) collagen-coated 96-well silicone substrates, and cell-tractile forces were measured at baseline (0 min) and after treatment (15, 30/60 min). Although thrombin (1 unit/mL), VEGF (100 ng/mL), and H₂O₂ (100 nM) increased baseline forces in a time-dependent manner, the rho kinase inhibitor Y27632 (10 μM) ablated them. Bevacizumab (0.05 mg/mL) prevented the VEGF-induced force increase. Plotted is the mean ± standard error pooled from 8–24 wells per time point per treatment. * indicates significant difference compared to baseline.

and uniform, and the fabrication of multiwell silicone substrates eliminates time-consuming production steps, utilizes standard microfabrication procedures, and obviates the need for surface-bound fluorescent beads by embedding them as a monolayer by spin coating. Moreover, silicone elastomers possess many material-favorable properties over PAA. Specifically, they are predominantly elastic in the stiffness range that encompasses most physiological microenvironments, are nonporous and impermeable—thus obviating common concerns associated with PAA (19)—and possess superior optical properties.

Mechanical malfunction appears to be an integral component of many diverse diseases, including asthma, ocular pathologies, acute lung injury, bladder dysfunction, vascular diseases, fibrosis, and cancer, wherein cell contractile forces play a pivotal role. CFS using elastic silicone substrates is expected to enable mechanistic studies for both quantitatively describing the aberrant contractile forces as well as mechanically rectifying the responses by directly evaluating potential therapeutic compounds.

SUPPORTING MATERIAL

Supporting Materials and Methods, nine figures, and one table are available at [http://www.biophysj.org/biophysj/supplemental/S0006-3495\(18\)30439-9](http://www.biophysj.org/biophysj/supplemental/S0006-3495(18)30439-9).

AUTHOR CONTRIBUTIONS

H.Y., N.K., R. Kaviani, and M.T. fabricated and measured PDMS TFM surfaces. K.R., S.Y., and Q.D. performed TFM in ASM cells. C.L. and J.K.S. optimized TFM in ASM cells. A.H. performed TFM in RPE cells. M.S.-G., R. Krishnan, and A.J.E. devised study, contributed reagents, and wrote the manuscript.

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SUPPORTING CITATIONS

References (33–36) appear in the Supporting Material.

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