

1 Quantifying Force Transmission through Fibroblasts:

2 Changes of Traction Forces under External Shearing

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13 ABSTRACT

Mammalian cells have evolved complex mechanical connections to their microenvironment, including focal adhesion clusters that physically connect the cytoskeleton and the extracellular matrix. This mechanical link is also part of the cellular machinery to transduce, sense and respond to external forces. Although methods to measure cell attachment and cellular traction forces are well-established, these are not capable of quantifying force transmission through the cell body to adhesion sites. We here present a novel approach to quantify intracellular force transmission by combining microneedle shearing at the apical cell surface with traction force microscopy at the basal cell surface. The change of traction forces exerted by fibroblasts to underlying polyacrylamide substrates as a response to a known shear force exerted with a calibrated microneedle reveals that cells redistribute forces dynamically under external shearing and during sequential rupture of their adhesion sites. Our quantitative results demonstrate a transition from dipolar to monopolar traction patterns, an inhomogeneous distribution of the external shear force to the adhesion sites as well as dynamical changes in force loading prior to and after the rupture of single adhesion sites. Our strategy of combining traction force microscopy with external force application opens new perspectives for future studies of force transmission and mechanotransduction in cells.

Introduction

Cells exert forces to interact with their surroundings and have the striking ability to react to externally applied forces and mechanical cues by a process called mechanotransduction¹⁻³. Cellular reactions to external mechanical cues play a crucial role in cellular processes such as stem cell differentiation, adhesion, migration, and proliferation⁴⁻⁸. Furthermore, focal adhesion clusters grow in response to external shearing^{9,10} which might help cells to withstand shear forces, e.g. forces exerted by the blood flow on endothelial cells^{11,12}.

Traction force microscopy (TFM) has become an established tool to quantify forces exerted by single cells or cell layers to the underlying substrate, which has deepened our understanding of cell migration, mechanotransduction and cell-matrix interaction¹³⁻²⁰. However, current traction force microscopy models assume an equilibrium of a cell's traction forces, whereas in nature cells experience a variety of externally applied forces, for instance from blood flow, muscle contraction, movement of other cells, or wound

opening. Force transmission is particularly important in tissue formation and adaption²¹ as well as in collective cell migration, where many cells interact with each other and mechanically strong cells become leader cells^{19,22}. Thus, to understand force transmission by cells more completely, it is crucial to study traction forces under external forces.

Techniques to exert mechanical stimuli to cells include atomic force microscopy (AFM), which can be employed to measure forces necessary to rupture cellular adhesions^{23,24} or forces exerted by cells^{25,26}, hydrodynamic shear stress^{11,12,27}, optical or magnetic tweezers^{3,28–30}, microneedle assays^{9,10,31} and optical stretchers^{32,33}. Despite the fact that such a large variety of physical cell manipulation techniques has been established and cellular forces exerted to surfaces can be measured via TFM or elastic resonator interference stress microscopy¹⁶, a quantification of cellular force adaptation as a response to well-defined mechanical stimuli applied to cells has not yet been realized.

Here, we present a new tool that combines TFM with externally applied mechanical stimulation by microneedle shearing. This setting allows to quantify cellular force transmission by measuring how cells distribute an external well-defined shear force to their adhesion sites. The spring constant of the microneedle is calibrated and thus the shear force exerted by the needle is known. We advanced current TFM procedures to create a novel procedure that analyzes traction forces in the presence of an external force monopole. This new force transmission assay is a versatile technique that is complementary to existing methods, as it can also be combined with other techniques such as AFM to broaden our understanding of the interplay of cellular biomechanics and adhesion.

Results and Discussion

To investigate the force transmission from the apical to the basal side of an adherent cell, we conducted experiments during which we exerted well-defined shear forces to the apical side of mouse embryonic fibroblasts (MEFs) while simultaneously measuring the change in traction forces at their basal side. We employed MEF fibroblasts expressing mNeonGreen (NeonG) labeled zyxin as a marker for focal adhesions. Cells were allowed to spread on a fibronectin-functionalized polyacrylamide (PAAm) gel with embedded red fluorescent marker beads so that traction forces could be derived from recording the displacement of the marker beads. A microneedle was installed into a micromanipulator such that the tip of the microneedle

54 was parallel to the cell substrate. Moving the microneedle with a computer-controlled micromanipulator
 55 results in the application of a shear force to the apical cell surface. The spring constant of this microneedle
 56 was calibrated by shearing polydimethylsiloxane (PDMS) pillars prior to the cell experiments. To do so,
 57 first the Young's modulus of the PDMS sample was measured with an AFM-based indentation method³⁴
 58 (see Supplementary Information). Then, the calibration of the microneedle was carried out by moving a
 59 microneedle against a PDMS pillar (Fig. 1) and measuring the associated PDMS pillar and microneedle
 60 bending. In A and B, representative phase contrast images of the shearing of a PDMS pillar with a
 61 microneedle show the bending of pillar and microneedle due to shear forces. Knowing the geometry as
 62 well as the Young's modulus of the pillar, the shear force is calculated from the pillar bending³⁵. C shows
 63 a plot of the shear force versus the microneedle bending for each frame of the experiment. The slope of a
 64 linear fit to this curve corresponds to the microneedle's spring constant.

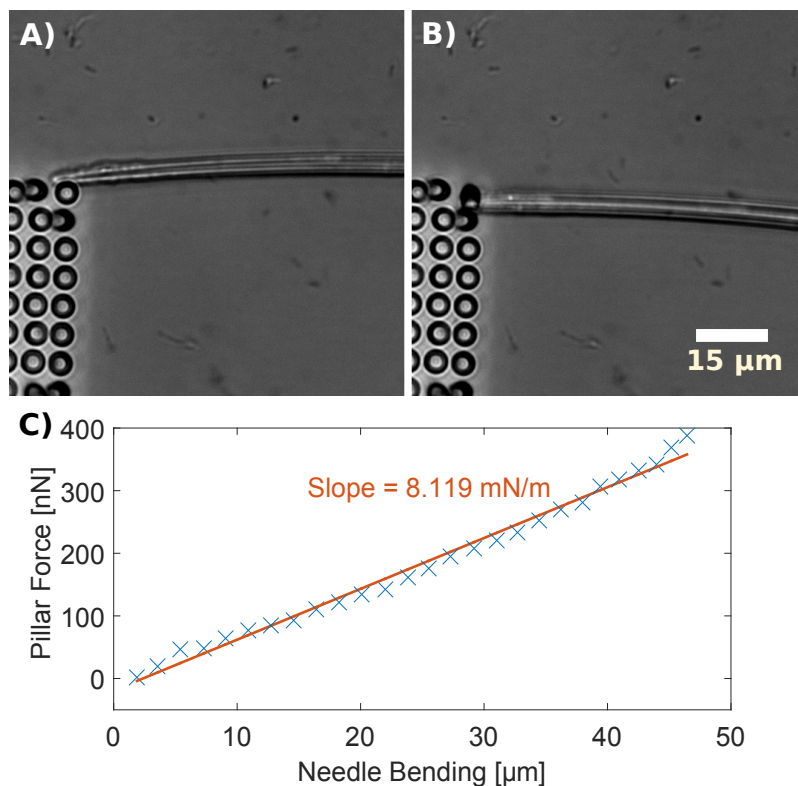


Figure 1. A and B show exemplary phase contrast images of a microneedle shearing a PDMS pillar. The needle moves downwards and bends the pillar. The pillar, on the other hand, exerts a force to the needle, which results in a bending of the needle. The force acting between needle and pillar is calculated from the bending of the PDMS pillar. C presents a plot of the pillar force versus the bending of the microneedle for each frame of the shearing experiment. The slope corresponds to the microneedle's spring constant.

65 For the force transmission experiments, the microneedle was carefully inserted into the fibroblast cell
66 directly below or above the nucleus. Subsequently, the microneedle was moved at a constant speed of
67 5 $\mu\text{m/s}$ towards the nucleus to exert increasing shear forces to the cell until the cell detached from the
68 underlying PAAm substrate. We decided to shear the nucleus, as other modes of exerting shear forces to
69 the cell caused the microneedle to quickly slip away. This is in agreement with published work by Riveline
70 et al.⁹ and Paul et al.¹⁰ who have shown that nucleus shearing is the most efficient way to transmit forces
71 to a cell with a microindenter. During the shearing process, phase contrast images of the cell and needle as
72 well as fluorescent images of the marker beads embedded in the PAAm gel were recorded. Fig. 2 A and B
73 present exemplary phase contrast images of such an experiment. A video containing all phase contrast
74 images as well as another video of the fluorescent marker beads are presented in the Supplementary
75 Information. These phase contrast images were used to monitor the cell as well as to calculate the degree
76 of needle bending for each frame. Knowing the needle's spring constant, the needle bending is a measure
77 for the shear force exerted to the cell. To correlate the measured traction forces with the distribution of
78 adhesion sites, the zyxin distribution of the fibroblast prior to each experiment was recorded (Fig. 2 C).
79 This information is essential, as focal adhesions are the main site of traction force exertion^{17,18}.

80 The images of the fluorescent marker beads embedded in the PAAm sample to which the cell is
81 adhering were used as the basis for computing the traction forces that the cell exerted to the PAAm sample
82 as a function of external shear force. As the microneedle applies external shear forces to the cell surface,
83 cellular traction forces are no longer balanced by internal forces only, and the overall force balance has to
84 include the overall force applied by the microneedle. In other words, the cell traction is not dominated by
85 the force dipole contribution, as it is usually the case, but also includes a force monopole. Our experimental
86 setup therefore requires several modifications to the force reconstruction algorithms commonly used in
87 TFM^{15,36}. Due to the existence of a force monopole, deformation is very long-ranged and boundary effects
88 must be considered. In Fourier space, the $k = 0$ mode becomes relevant, which cannot be reconstructed
89 with the standard Fourier Transform Traction Cytometry (FTTC) procedures due to the divergence of the
90 Green's function at $k = 0$. Finally, TFM usually uses the inverse method which requires regularization,
91 but this procedure tends to underestimate absolute force values, which are especially important in our
92 context³⁶.

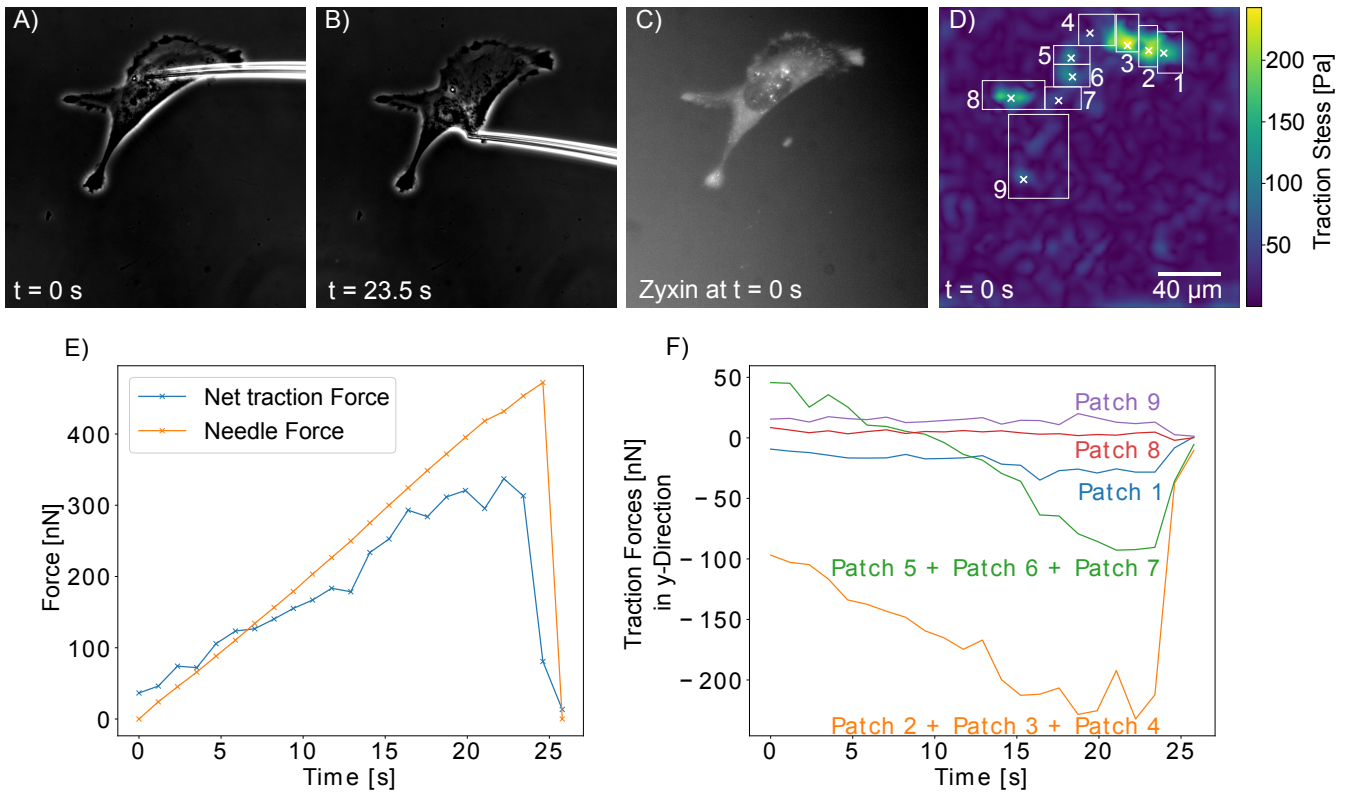


Figure 2. A microneedle is inserted into a fibroblast, which expresses fluorescently labelled zyxin and adheres to a TFM substrate. Subsequently, the needle is moved into the y-direction at a constant speed and exerts shear forces to the cell until it is detached. A) and B) Exemplary phase contrast images taken during cell shearing. Both the cell and the needle bending are monitored. The bending of the needle is used to calculate the shear force. C) The cell's zyxin distribution prior to the shearing process is recorded via fluorescence microscopy. D) Traction force map of the cell with adhesion search areas delimited by white rectangles and mean patch locations marked by crosses. Traction forces were reconstructed for $t = 0$ s using Fourier Transform Traction Cytometry (FTTC). For the reconstruction of traction forces with the shear force monopole present ($t > 0$), we used the circular patch method. E) The needle force and the cell's net traction force are plotted as a function of time. F) The traction forces in y-direction are plotted for different adhesion patches (labelled in panel D) to quantify how the cell loads its adhesion sites under the external shearing stimulus.

Due to these limitations, we avoided Fourier space methods^{17,37} and worked directly in real space. Although continuous force distributions can in principle be reconstructed with the boundary element method (BEM)^{38,39}, here we make additional use of the fact that the cells used in our experiments have well-defined adhesion sites that are increasingly stressed as the cell is sheared by the microneedle. Motivated by this observation, we use a method where localized forces are distributed inside the cell contour⁴⁰⁻⁴³. Rather than using point forces⁴⁰, which also suffer from the divergence problem, we use known contact mechanics solutions for traction forces transmitted on circular areas^{44,45}, for which the

100 divergence of the Green's function is removed by integration over the contact region. The adhesion
101 forces are estimated using the known deformation-force relation for a constant traction applied over a
102 circular area. Recent studies have suggested that adhesion sites have in fact more elliptic shapes^{36,40,46–48}.
103 This does, however, not have a significant impact on the force reconstruction (see the Supplementary
104 Information for a more thorough discussion). By summing over all adhesion sites and minimizing the
105 deviation between experimental and estimate deformations, one arrives at the theoretical estimate for the
106 traction force field (see the Supplementary Information for more details).

107 Fig. 2 D shows a traction force map computed with our method. Our algorithm first determines
108 the main sites of traction force transmission from the cell to the PAAm sample. These sites ("adhesion
109 patches") are marked by white crosses and numbers in the figure panel. We calculated the traction force
110 vector of each adhesion patch and then determined the magnitude of the sum of all traction force vectors.
111 This net traction force magnitude was then compared to the needle force. As traction forces without a force
112 monopole are balanced, introducing an externally applied force must result in a change of traction forces
113 to balance the externally applied force. Our results shown in Fig. 2 E demonstrate that the net traction
114 force and the externally applied shear force closely matched during the entire experiment, validating our
115 analysis approach. The fact that the shear force and net traction force do not match perfectly might have
116 several reasons: force can be dissipated²⁴ or cells might resist deformation with cell specific responses.
117 Furthermore, as several calibration steps are needed during force calculations, our results are prone to
118 calibration errors: The needle spring constant was calibrated via shearing a PDMS pillar and the Young's
119 modulus of the PDMS was measured for the computation of the needle's spring constant. Furthermore,
120 the PAAm's Young's modulus needed to be determined in order to reconstruct traction forces from the
121 bead displacement data. Both materials' elastic properties were measured with a state-of-the-art atomic
122 force microscopy procedure³⁴ naturally prone to measurement errors, which means that neither the needle
123 force, nor the traction forces are perfectly accurate. Image analysis inaccuracies in the quantification of
124 the needle bending and bead displacement may further contribute to the slight mismatch between the net
125 traction force and externally applied force.

126 In Fig. 2 F, the y-components of traction force vectors are plotted for the different adhesion patches. For
127 better visualisation, we combined some neighbouring adhesion patches with similar force loading behavior.

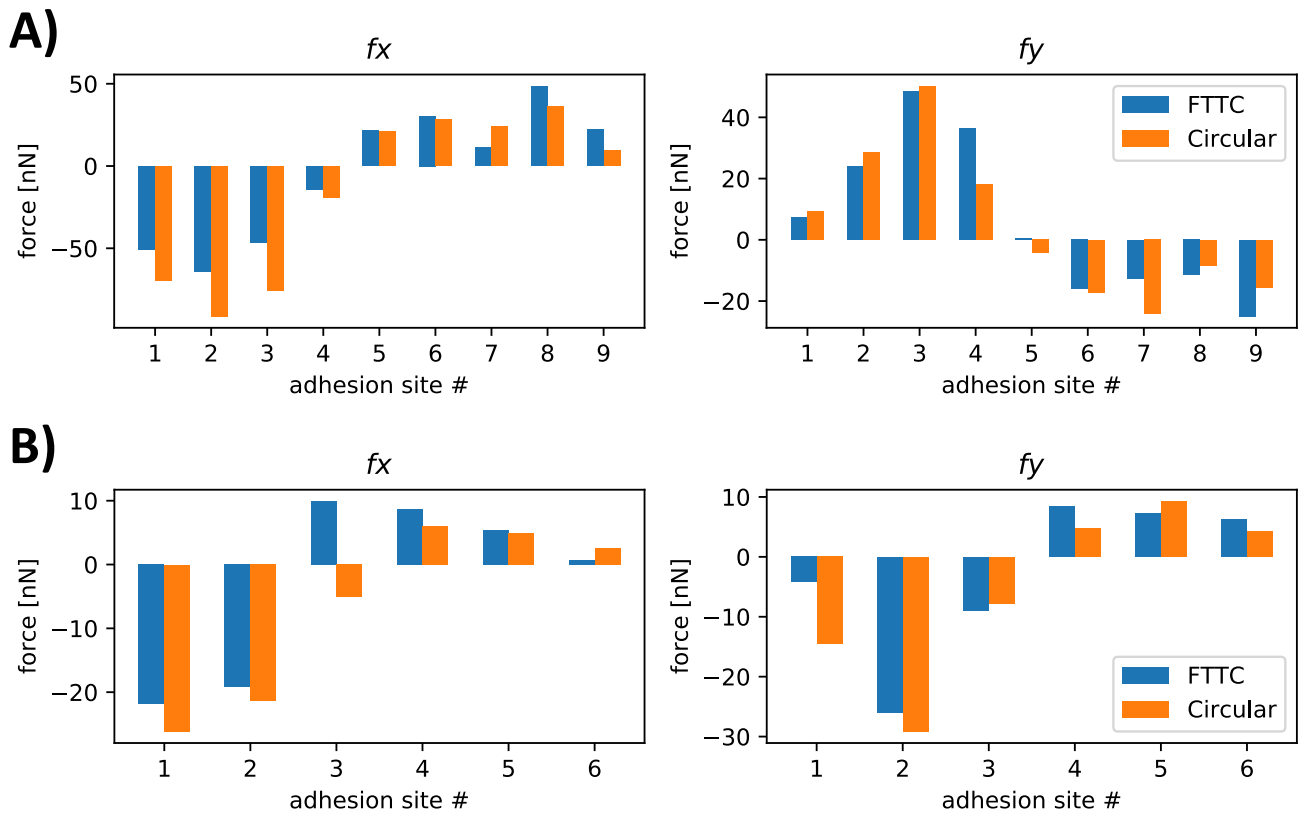


Figure 3. Comparison of the traction forces predicted in the absence of an external force monopole (at $t = 0$) using the circular patch method (that we employed during this study) and a regularized Fourier Transform Traction Cytometry (FTTC)¹⁴ using generalized cross-validation⁴⁹. A) Profile for the cells introduced in Fig. 2. B) Profile for the cells introduced in Fig. 5. In both cases, the agreement between the two methods is rather good.

128 The data for each individual adhesion patch is presented in Fig. 5 of the Supplementary Information. As
 129 the needle pulled mainly in the y-direction, the x-components of the traction vectors were not influenced
 130 by the needle shear force, which is why we concentrate on discussing the y-components (a graph of the
 131 x-components of the traction vectors are presented in the supplementary information). One sees that
 132 the microneedle pulling mainly loads the adhesion patches 2, 3 and 4, and to a lesser extend also the
 133 adhesion patches 5, 6 and 7. This result had to be expected due to the position of these adhesions in the
 134 part of the cell that is tensed by the needle. On the other hand, Patches 8 and 9 are not or only slightly
 135 loaded, presumably because they are located in the part of the cell subjected to compressive forces during
 136 needle shearing. The plot shows that loading is not homogeneous and most likely is related to cytoskeletal
 137 elements (e.g. between adhesions and nucleus) not visible here. The asymmetric response of different

138 adhesion patches can be explained by the fact that the cytoskeleton is made from semiflexible polymers,
139 which respond differently to pulling and pushing. Pulling reduces entropy and increases stretching as
140 well as bending energies, eventually leading to strain stiffening⁵⁰. Pushing, on the other hand, meets
141 little resistance, because cytoskeletal filaments tend to buckle under force and the cytoplasm can flow
142 away, thus it is difficult to locally build up compression energy like in a solid^{51,52}. It is interesting to
143 note that also in the physiological context, cell mechanics is probed mainly in pulling, not in pushing,
144 e.g. in epithelial monolayers, which are under large prestress⁵³. Therefore, pulling is the relevant mode
145 and much more meaningful than pushing. Thus, patches 2 - 7 were loaded presumably because the
146 needle pulling forces were transmitted efficiently to these adhesion patches through the polymers of the
147 cytoskeleton. Correspondingly, patches 8 and 9 were probably not loaded because pushing forces are not
148 transmitted well by cytoskeletal polymers⁵⁴. This is in agreement with earlier studies^{9,10,37}, but our results
149 quantify the traction forces for individual focal adhesion patches under an external mechanical stimulus
150 in an unprecedented way. Our findings also demonstrate the complexity and non-uniform distribution of
151 intracellular force transmission as a function of load and location.

152 The good agreement between the needle force and the net traction force predicted with our circular
153 patch method shown in Fig. 2 E is a first and successful validation of our approach. To further validate
154 it, we reconstructed forces at $t = 0$ (when there is no force monopole) at single patches with Fourier
155 Transform Traction Cytometry (FTTC) with 0th order Tikhonov regularization¹⁴, where the regularization
156 parameter is determined by generalized cross-validation⁴⁹. Adhesion forces were then calculated by
157 integrating the traction stress in each search window, both for the cell analyzed in Fig. 2 and the cell
158 analyzed in Fig. 5. As shown in Fig. 3, the agreement between the two methods is rather good in both
159 cases.

160 Because an unperturbed cell to lowest order forms a force dipole, while the needle presents a force
161 monopole, we next calculated the force moments as a function of time. Because momentum and also
162 angular momentum is not conserved anymore due to the external pulling, one has to be careful how to
163 define these moments (explained in Supplementary Information). Fig. 4 A shows that for the cell shown in
164 Fig. 2, the monopole increases with time, but the major dipole does not decrease as expected. The torque
165 remains low but shows a slight upwards slope. The explanation is provided by Fig. 4 B, which explicitly

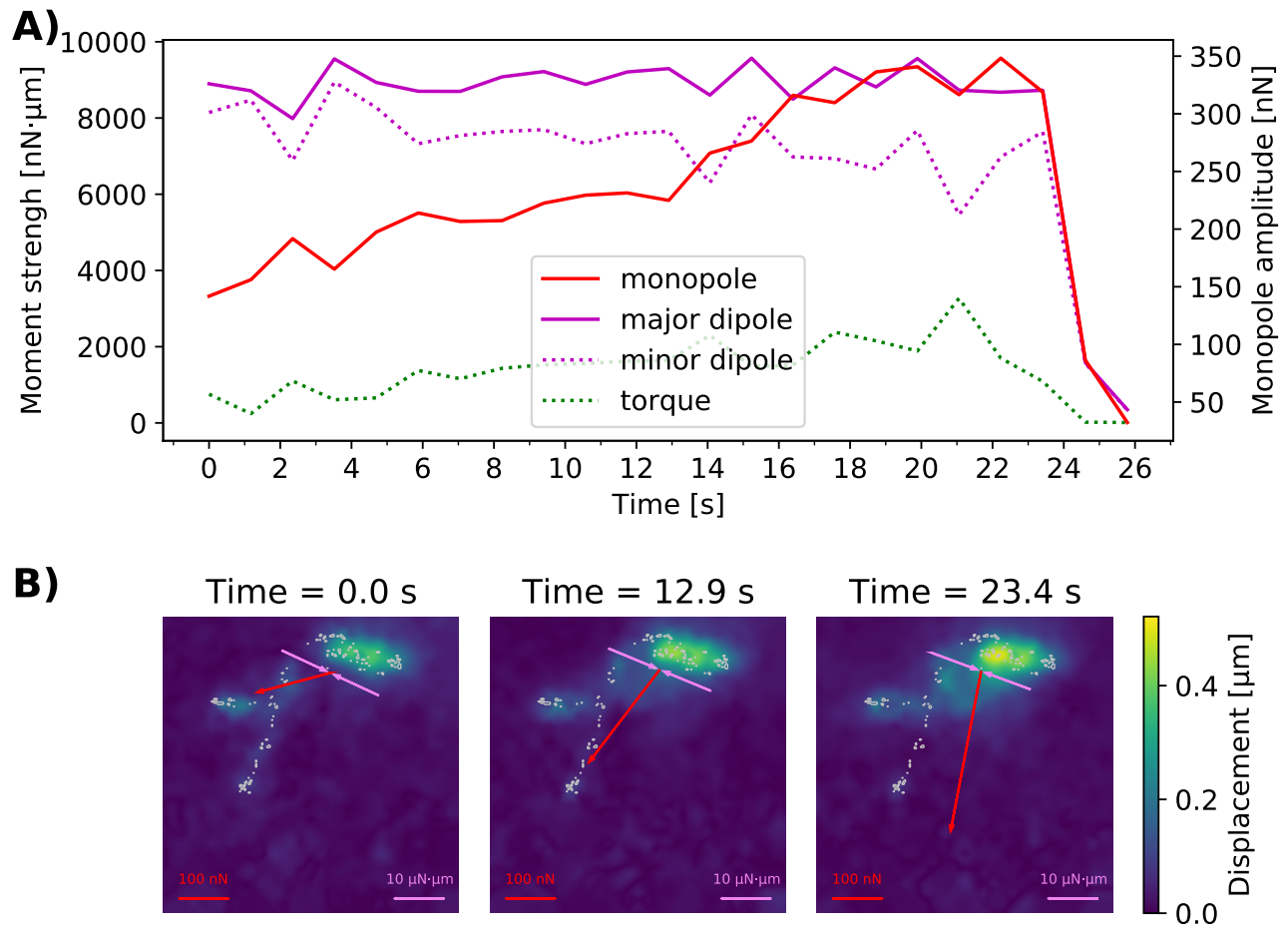


Figure 4. Change of force monopole and dipole moments of the cell presented in Fig. 2 in response to needle shearing. A) presents the magnitudes of the force monopole, as well as the major and minor dipole moments and the torque as functions of time. Our results show that the contractile forces are initially distributed mostly isotropically around the contractile center. However, the force monopole created by the needle shearing increases over time while the minor dipole, which describes the contractility in the direction of the force, decreases slightly. B) shows the force monopole and the major dipole moment in exemplary force maps recorded during the shearing experiment. The force monopole is denoted by red arrows while the dipole moment is represented by purple arrows. The gray encircled regions represent areas where adhesions are predicted from the cell's zyxin distribution.

shows the monopole (in red) and the major dipole (in purple). Because they are oriented perpendicularly to each other, the microneedle pulling does not perturb the cellular dipole for a long time, until complete failure occurs.

We now turn to an example in which monopole and dipole orientations are co-linear. For the cell presented in Fig. 5, only adhesion patches 1, 2 and 3, which - in contrast to patches 4, 5 and 6 - are loaded

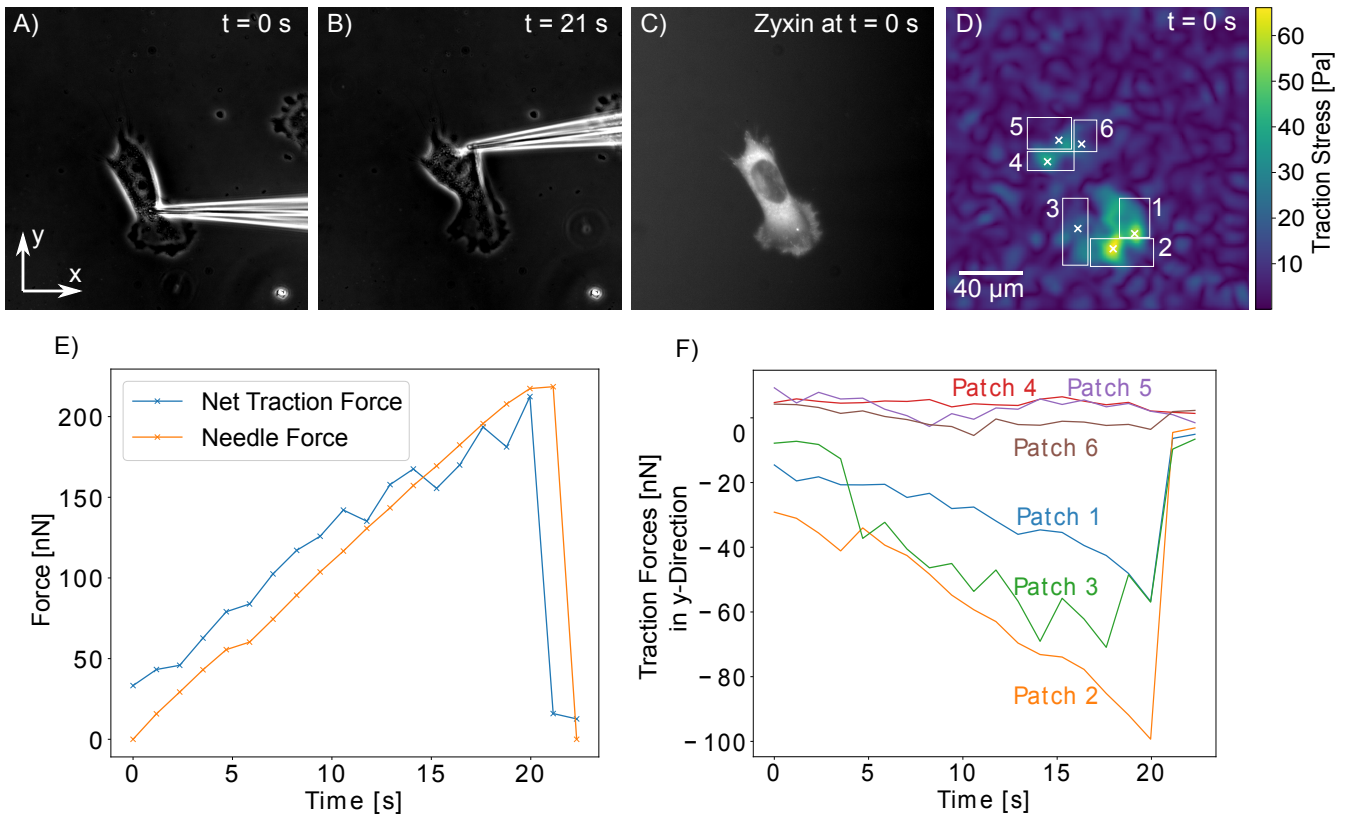


Figure 5. The change of traction forces as a response to microneedle shearing. A) and B) show phase contrast images of a cell adhering to a PAAM substrate and a microneedle exerting shear forces to the cell. The cell's zyxin distribution prior to the shearing process is presented in C) while D) pictures a traction force map with cell's adhesion patch positions marked with white crosses and numbers. The force map is calculated using FTTC at $t = 0$ s.

In E) The shear force exerted by the needle to the cell is compared to the magnitude of the net traction force vector. The y-components of the traction vectors for the adhesion patches are plotted for each moment of the experiment in F).

171 in tension, experience an increase in their traction forces. Furthermore, the traction forces exerted through
 172 patch 2 change most strongly. This is another indication that force components perpendicular to the shear
 173 force vector are not affected by the shearing process as patch 2 lies directly below the site of shear force
 174 exertion and thus has much weaker traction forces perpendicular to the shearing direction than patches 1
 175 and 3. Fig. 5 E shows that the total traction forces exerted through the cell have the same magnitude as the
 176 needle shear force, which confirms the validity of our approach. In Fig. 6 we plot the force monopole
 177 as well as the major and minor dipole moments measured during the experiment presented in Fig. 5 as
 178 functions of time. These data demonstrate that the force balance changes from a situation that is governed
 179 by the major dipole moment to one dominated by the force monopole that is created by the needle shearing.

180 While the adhesions in front of the needle are less exposed to the stress, the ones behind are subjected to
 181 large tensile cytoskeletal forces. Interestingly, the cellular dipole becomes more and more localized to the
 182 tensed region, indicating a strong reorganization or rearrangement also inside the cell. This is supported
 183 by the torque that experiences a downward slope indicating that the adhesive center becomes more aligned
 184 with the microneedle.

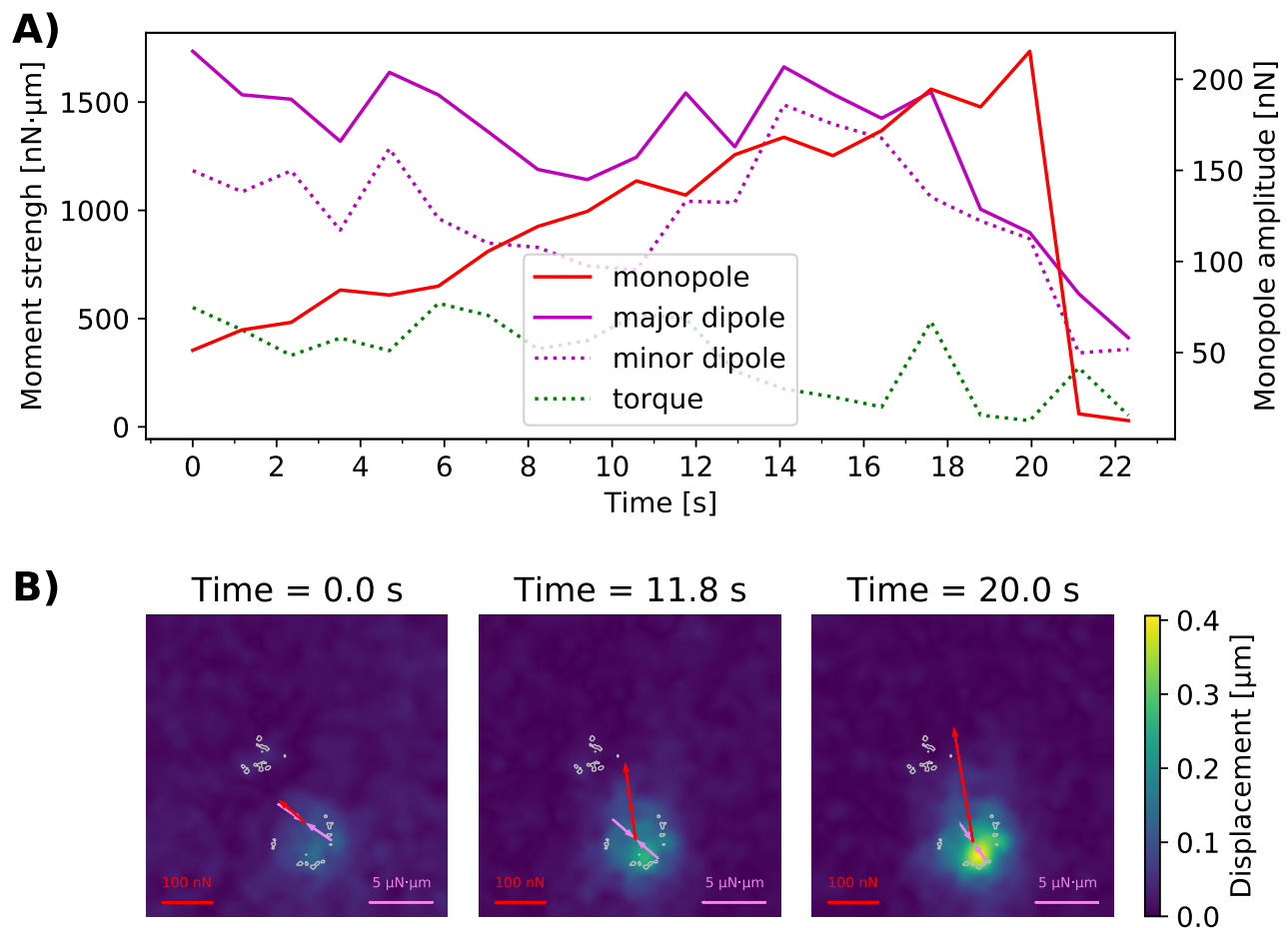


Figure 6. Change of force monopole and dipole moments of the cell presented in Fig. 5 in response to needle shearing. A) presents the magnitudes of the force monopole, as well as the major and minor dipole moments and the torque as functions of time. Our results show that the force balance is initially governed by the major dipole moment. However, the force monopole created by the needle shearing increases over time and governs the force balance at high shearing forces. B) shows the force monopole and the major dipole moment in exemplary force maps recorded during the shearing experiment. The force monopole is denoted by red arrows while the dipole moment is represented by purple arrows. The gray encircled regions represent areas where adhesions are predicted from the cell's zyxin distribution.

185 The results presented in Fig. 7 show once more that not all adhesion patches are loaded with forces.

186 Patches 1 as well as 4 and 5 were not loaded under an external shear force. Interestingly, not only patches
 187 2 and 3, which were closest to external force application site were loaded, but also adhesion patches
 188 10 and 11, even though they were further away from the needle than patches 1, 4 and 5. These data
 189 suggest that internal transmission of tension can be long-ranged, for example through stress fibers, as
 190 recently demonstrated by optogenetic control of cell contractility⁵⁵. In order to analyse this important
 191 aspect in detail, future work has to simultaneously image also the actin cytoskeleton. However, this is very
 192 challenging, as we also have to image the zyxin-marked focal adhesions and the fluorescent marker beads
 193 in the elastic substrates.

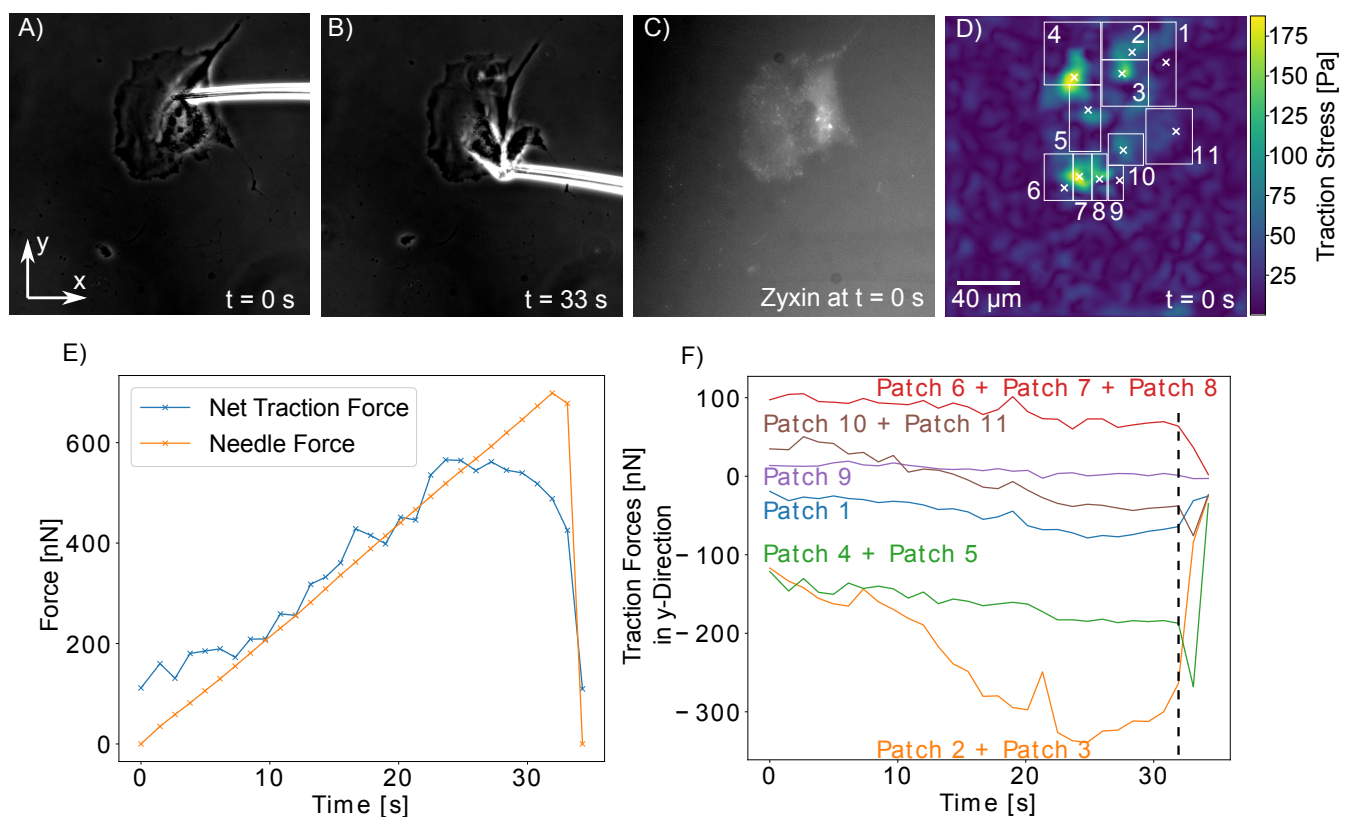


Figure 7. Redistribution of adhesion patch loading after a rupture event. A) and B) show phase contrast images of a microneedle shearing a fibroblast on a PAAm substrate. The cell's zyxin distribution is visualized in C). A map of the traction forces at $t = 0$ s exerted at the cell's adhesion patches is presented in D). The white crosses mark the cell's adhesion sites. The force map is calculated using FTTC at $t = 0$ s. The sum of traction forces has roughly the same magnitude as the external shear force, as can be seen in E). The y-components of the traction vectors for the adhesion patches are plotted in F). The dashed line marks the rupture of adhesion patches 1, 2 and 3 at $t = 32$ s

194 It is a well-established fact that focal adhesions rupture successively under external forces²⁴, nonethe-
 195 less our results presented in Fig. 7 quantify for the first time the redistribution of traction forces throughout

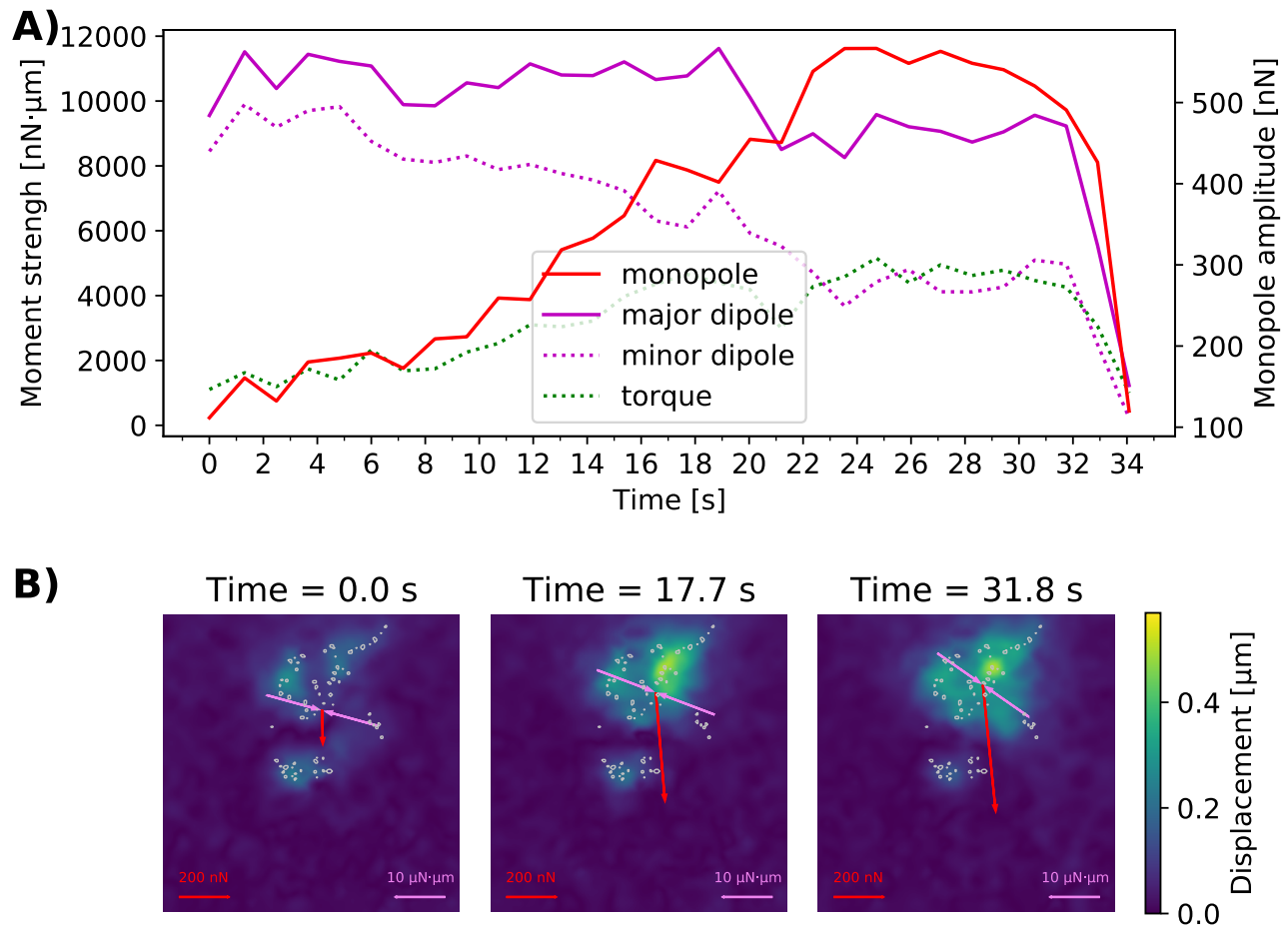


Figure 8. Change of force monopole and dipole moments of the cell presented in Fig. 7 in response to needle shearing. A) presents the magnitudes of the force monopole, as well as the major and minor dipole moments and the torque as functions of time. Our results show that the force balance is initially governed by the major dipole moment. However, the force monopole created by the needle shearing increases over time and governs the force balance at high shearing forces. B) shows the force monopole and the major dipole moment in exemplary force maps recorded during the shearing experiment. The force monopole is denoted by red arrows while the dipole moment is represented by purple arrows. The gray encircled regions represent areas where adhesions are predicted from the cell's zyxin distribution.

196 the cell after the rupture of adhesion sites. When adhesion patches 1, 2 and 3 ruptured after 32 s (marked
 197 by the dashed line) - even though adhesion patch 1 had barely been loaded with force before that - the
 198 traction forces exerted through all other patches except patch 9 increased substantially. Interestingly,
 199 patches 10 and 11, which had been the only patches that were loaded strongly prior to the rupture event,
 200 were only loaded with a small amount of force upon the rupture event, while patches 4 and 5, which had
 201 been only marginally loaded, changed their traction forces much more strongly following the rupture

event. In the future, one might use adhesive micropatterns to control the exact location of the adhesion patches and therefore the way individual adhesion sites are loaded by the shearing force.

In Fig. 8 we present the force monopole as well as the major and minor dipole moments measured during the experiment presented in Fig. 7 as functions of time. The behavior is similar to the one presented in Fig. 6.

Another striking aspect is the fact that the traction forces exerted through patches 2 and 3 started to slowly decrease several seconds prior to the rupturing event. We observed a similar behavior for adhesion patch 3 of the cell presented in Fig. 5. This suggests that the rupture of focal adhesions is not necessarily an instantaneous event, but that there exist rupture processes of extended duration, which we recorded using our novel analysis approach. Strikingly, the load on some focal adhesions decreased prior to rupture while in others, the traction forces increased until they ruptured. Similar differences in adhesion site behavior have been described before as slip bonds and catch bonds⁵⁶, but in our experimental setting, which analyzes the behaviour of intact cells, the mechanical properties of the cell and force transmission through the cytoskeleton likely play an important role, too. Our new technique hence enables us to reveal possible physical factors that influence dynamic changes in force loading of adhesion sites.

Conclusion

We have introduced a novel method for determining traction forces in cells under external shear forces. The applicability of our method has been proven by shearing fibroblasts off their underlying PAAm substrates while monitoring the change in cellular traction forces at specific adhesion sites. We have shown that cells on soft substrates distribute an external shear force non-uniformly among their adhesion sites, as a function of location and load (tensile vs. compressive). Notably, we found that force transmission can be long ranged and mainly applies to adhesions that are under tensile load. This result may be due to the polymeric nature of the cytoskeletal network, which is better suited for the transmission of tensile forces. As our technique monitors the change in traction forces simultaneously to the shearing stimulation, it introduces a new quality to the recordings of rupture events to complement conventional techniques such as the single-cell force spectroscopy. Indeed, our method can be easily adapted to other force exertion methods and hence is very versatile and complementary to existing procedures. In the future, it could

229 be combined with imaging of the cytoskeleton to achieve a more complete understanding of how force
230 is transmitted through the cell. Moreover adhesive micropatterns might be used to better control the
231 positioning of the adhesion patches; with these two elements in place, we expect that our method can be
232 used to achieve a comprehensive understanding of how force is transmitted through adherent cells.

233 **Materials and Methods**

234 **Cell Culture**

235 Mouse embryonic fibroblasts expressing mNeonGrenn labeled zyxin were cultured at 37°C in 21% O₂,
236 5% CO₂ at a humidity of 95%. Dulbecco's Modified Eagle Medium (DMEM; Biochrom) containing 10%
237 fetal bovine serum (FBS; Biochrom) and 1% penicillin/streptomycin (pen strep; 10.000 µg/ml; Biochrom)
238 served as cell culture medium. Cells were seeded on a PAAm sample by removing the cell culture medium
239 and rinsing the cells at a confluency of about 80 % with PBS. The cells were incubated in trypsin/EDTA
240 (0.5%/0.2% in 10xPBS; Biochrom) at 37°C for 1 minute. Cell culture medium was added to stop the
241 trypsination process and the cells were separated from the liquids via 5 min of centrifugation at 2412 rpm.
242 The supernatant was replaced by fresh cell culture medium. The cells were redispersed and 100 µl of cell
243 suspension added to a PAAm sample with another 900 µl of DMEM. The cells were allowed to spread on
244 the PAAm sample overnight at 37°C prior to shearing experiments.

245 **Polyacrylamide Preparation**

246 A ∅ 50 mm FluoroDish Cell Culture Dish (World Precision Instruments) was pretreated to promote PAAm
247 attachment. They were cleaned three times with ethanol and double-distilled water before being incubated
248 in sodium hydroxide (NaOH, 2.5 M) for 10 min. Subsequently, the slides were cleaned in an ultrasonic
249 bath in double-distilled water for 10 min, rinsed with ethanol and incubated for 15 min in a mixture of
250 97 % ethanol (absolute), 2 % 3-(Trimethoxysilyl)propyl methacrylate (Sigma-Aldrich) and 1 % acetic acid
251 (Sigma-Aldrich). Subsequently, they were rinsed with ethanol and dried in air. A marker bead solution
252 was prepared by adding 100 µl fluorescent beads (1% solids, nominal ∅ 50nm, Flash Red, Bangslabs,
253 Cat. No. FSFR001) in 900 µl double-distilled water. The solution was cleaned twice by centrifuging and
254 replacing of the supernatant with double-distilled water.

255 PAAm was produced by degassing a solution of 150 acrylamide μl (40%; Biorad), 90 μl bis-acrylamide
256 (bis; 2%; Biorad), 10 μl 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid buffer (HEPES; 0.5 mM;
257 pH = 7; Biochrom), 255 μl aqueous Acrylic acid N-hydroxysuccinimide ester solution (2 wt%, Sigma-
258 Aldrich, CAS No 38862-24-7), 3 μl NaOH (2.5 M), and 10 μl marker bead solution in vacuum for 20 min.
259 Subsequently, 2.5 μl ammoniumperoxodisulfate (10 wt% in aqueous solution; Sigma-Aldrich; CAS No
260 7727-54-0) and 0.375 μl N,N,N',N'-Tetramethylethylenediamine (TEMED, Sigma-Aldrich, CAS No
261 110-18-9) were added to 260 μl of the acrylamide solution. After thoroughly mixing the solution, 10 μl
262 were deposited into a pretreated FluoroDish and covered with a round $\varnothing$ 18 μm coverslip. The sample
263 was left to polymerize in darkness for 30 min before the coverslip was removed. The sample was soaked
264 in double distilled water for 3 days. The water was removed and the sample was incubated in 100 μl
265 fibronectin (aqueous 100 $\mu\text{g}/\text{ml}$ solution) overnight at 6°C. The sample was shaken in 70% ethanol for
266 10 min and rinsed three times with sterile double-distilled water. Cells were added and allowed to spread
267 overnight prior to shearing experiments. Young's modulus of exemplary PAAm samples were measured to
268 be 16.5 ± 0.5 kPa employing our priorly published procedure³⁴. Details are published in the supplementary
269 information.

270 **Microneedle Preparation**

271 Microneedles were pulled from hollow borosilicate glass tubes (outer diameter 1 mm, inner diameter
272 0.5 mm, length 100 mm, item #: B100-50-10, Sutter Instruments Co.) using a Flaming/Brown micropipette
273 puller (Model P-97, Sutter Instruments Co.). The employed parameters were Pressure = 500, Heat = 490,
274 Velocity = 70, Pull = 70, Time = 100. For descriptions of these parameters please refer to⁵⁷. A MF-900
275 Microforge (NARISHIGE Group) was used to bend the needle so that the tip and base form an angle
276 of about 45°. Each needle was installed into a micromanipulator so that its tip is parallel to the sample
277 surface.

278 **Microneedle Calibration**

279 Each time a needle was installed to the micromanipulator, its spring constant was calibrated prior to cell
280 shearing experiments by shearing the needle against a PDMS pillar. Prior to calibration, a PDMS sample
281 with a network of 6 μm long pillars with a radius of 2 μm was soaked in water and degassed in vacuum for

10 minutes to remove air bubbles. The microneedle was positioned next to a PDMS pillar. The optimum needle height was determined by lowering the microneedle in 1 μm steps and trying to shear the pillar after each step to see if the needle slips before the pillar bends. Subsequently, the needle was moved against the pillar at 2 $\mu\text{m/s}$ while phase contrast images were recorded at a frame rate of 0.85 fps. At least one image of the unbent needle was acquired before the shearing process was initiated. This image served as a reference for calculation of needle bending. To eradicate errors from needle asymmetry, the direction of needle movement was chosen to be the same during cell shearing experiments and during the calibration process. To calculate the spring constant, the positions of the needle tip and the PDMS pillar were tracked manually in phase contrast images with imageJ. The respective positions in the reference frame were subtracted to compute the distances the tip had moved and the PDMS pillar had bent. For each frame, the time stamps of the phase contrast images were employed to calculate how much time has passed since the needle movement had started. This duration was multiplied with the speed of needle movement to compute the distance the needle had moved. The microneedle bending was calculated as the difference between micromanipulator distance and needle tip distance. The force necessary to bend a PDMS pillar was calculated as published by Schoen et al.³⁵. Young's modulus of the PDMS sample had been measured to be 801.5 ± 32.9 kPa employing our previously published procedure³⁴. The pillar force was plotted versus the needle bending and a linear fit is employed to calculate the slope of the resulting curve that corresponds to needles spring constant. We present an exemplary calibration experiment as well as details on the determination of the PDMS sample's Young's modulus in the supplementary information.

Shearing Process and Shear Force Calculation

A fluorescence image of the zyxin distribution of a well-spread fibroblast was recorded. The calibrated microneedle was inserted into this cell directly above or below the nucleus and a phase contrast image of cell and needle was recorded. Subsequently, the needle was moved horizontally at 5 $\mu\text{m/s}$ against the nucleus. During the shearing process, phase contrast images of cell and needle as well as fluorescent images of the marker beads embedded in the underlying PAAm substrate were recorded alternately at a frame rate of 0.85 fps. After cell detachment, an additional pair of fluorescent microscopy and phase contrast images was recorded. This last fluorescent image pair recorded the bead position of the

309 PAAm sample without any influence of traction forces and served as reference image for traction force
310 calculations.

311 Images were recorded using an inverted microscope (Z1 Observer, Zeiss) equipped with a CMOS
312 Camera (Hamamatsu ORCA Flash 4.0) and a 40x objective with phase contrast (Zeiss EC Plan-Neofluar
313 40x/0.75 Ph2 M27). Both, the phase contrast images and the fluorescence images were recorded using
314 the RFP filtercube (necessary to image the fluorescent marker beads) to minimize the time between
315 the measurement of shear force and traction forces. The microneedle was handled using a Eppendorf
316 InjectMan NI2 micromanipulator. For each frame, the bending of the needle was calculated as described
317 in the calibration section above. The shear force was computed by multiplying the needle bending with
318 the needle's spring constant calibrated prior to each experiment.

319 **Calculation of Traction Forces**

320 Traction forces were calculated using a home-written algorithm that employs the established deformation-
321 force relation for a constant traction applied over a circular area. A single adhesion was assumed per
322 area. The location of each adhesion center within each area was determined for each frame using the local
323 maxima approach. The adhesion radius was found by inspecting the surrounding peaks. A common radius
324 was determined for each adhesion, which was then used for all frames. A detailed description can be found
325 in the supplementary information. The substrate deformation field was obtained from fluorescent bead
326 images using PIV^{58,59}. A window size of 64 pixels and a 50% window overlap was used. Spurious vectors
327 were removed using a minimal signal-to-noise ratio in the correlation function of 1.5 and a threshold of
328 2.0 for the normalized median test. The reference image was taken after cell detachment.

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Quantifying Force Transmission through Fibroblasts: Changes of Traction Forces under External Shearing - Supplementary Information

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Reconstruction of traction forces

In our analysis, we assume that focal adhesions, where force is transmitted to the substrate, have a circular shape. For the far field, it is not essential whether the traction forces within an adhesion are distributed evenly or vary over this area, for example decaying towards the rim. In any case, the far field would result in the Green's function of the elastic halfspace, which represents a point force in the middle of the adhesion [1]. For the near field, the solutions look slightly different. Therefore the main difference between different assumptions would be the reconstruction of the forces in the middle of the focal adhesions. To compare the effect of different assumptions, we initially consider two types of circular adhesive patterns: the constant traction force patterns and radial decreasing Hertz-like traction force pattern.

The analytical solution for the surface deformation created by a tangential traction force $\mathbf{F} = (F_x, F_y)^T$ distributed equally over a circular area with radius a at the surface of a sufficiently thick, substrate in the linear, isotropic elastic regime has recently been calculated in the context of traction force microscopy [2]. Employing polar coordinates $\mathbf{r} = r(\cos \theta, \sin \theta)$ centered around the middle of the circular adhesion, the surface traction profile is given by:

$$\boldsymbol{\tau}(r, \theta) = \begin{cases} \frac{\mathbf{F}}{\pi a^2} & r < a \\ 0 & r \geq a \end{cases} \quad (1)$$

The corresponding deformation field is given by

$$u_x(r, \theta) = \frac{1 + \nu}{\pi^2 a E} [(1 - \nu)N_1(r, \theta) + \nu N_2(r, \theta)]F_x - \nu N_3(r, \theta)F_y \quad (2)$$

$$u_y(r, \theta) = \frac{1 + \nu}{\pi^2 a E} [-\nu N_3(r, \theta)F_x + (1 - \nu)N_1(r, \theta) + \nu N_4(r, \theta)]F_y \quad (3)$$

Here the E describes the substrate stiffness (Young's modulus) and ν the Poisson ratio. The functions N_1 to N_4 have the following form in the inner region where

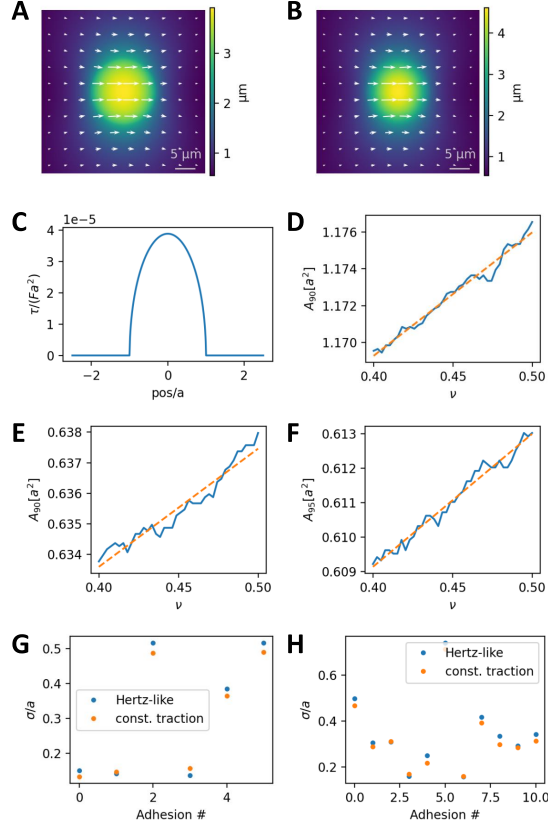


Figure 1: **A** Plot of the surface deformation created by a constant-traction profile with $a = 8\mu\text{m}$, $F = \pi a^2 \cdot 1.5\text{kPa}$, $E = 6.9\text{kPa}$, $\nu = 0.5$. **B** Plot of the surface deformation created by a Hertz-like profile, same parameters as in **A**. **C** cross-section for the Hertz like profile. **D** Numerical relation between A_{90} and ν for the constant-traction profile. **E** Same relation for the Hertz-like profile. **F** Numerical results relation between A_{95} and ν for the constant-traction profile. **G** Comparison of the relative standard deviation in the definition of areas for the cell presented in Fig. 5 of our main manuscript. **H** Same for the cell presented in Fig. 2 of our main manuscript.

$r < a$ and $\xi_1 = r^2/a^2$:

$$N_1 = 4E_0(\xi_1) \quad (4)$$

$$N_2 = \frac{4 \cos(2\theta) ((r^2 + a^2)E_0(\xi_1) + (r^2 - a^2)K_0(\xi_1))}{3r^2} + 4 \sin^2 \theta E_0(\xi_1) \quad (5)$$

$$N_3 = \frac{2 \sin(2\theta) ((r^2 - 2a^2)E_0(\xi_1) + (r^2 - a^2)K_0(\xi_1))}{3r^2} \quad (6)$$

$$N_4 = 4 \cos^2 \theta E_0(\xi_1) - \frac{4 \cos(2\theta) ((r^2 + a^2)E_0(\xi_1) + (r^2 - a^2)K_0(\xi_1))}{3r^2}. \quad (7)$$

Here, E_0 and K_0 describe the complete elliptic integral of the first and second kind, respectively. For the outer region where $r > a$ and $\xi_2 = a^2/r^2$ we have

$$N_1 = \frac{4 (r^2 E_0(\xi_2) + (a^2 - r^2) K_0(\xi_2))}{ar} \quad (8)$$

$$N_2 = \frac{(6r^2 - 2(r^2 - 2a^2) \cos(2\theta)) E_0(\xi_2) + 2(r^2 - a^2)(\cos(2\theta) - 3) K_0(\xi_2)}{3ar} \quad (9)$$

$$N_3 = \frac{2 \sin(2\theta) ((r^2 - 2a^2) E_0(\xi_2) + (a^2 - r^2) K_0(\xi_2))}{3ar} \quad (10)$$

$$N_4 = \frac{(6r^2 + 2(r^2 - 2a^2) \cos(2\theta)) E_0(\xi_2) - 2(r^2 - R^2)(\cos(2\theta) + 3) K_0(\xi_2)}{3ar}. \quad (11)$$

The shape of the deformation field is shown in Fig. 1.

In the limit $r \rightarrow 0$ we find that

$$\mathbf{F} = \frac{\pi a E}{(1 + \nu)(2 - \nu)} \mathbf{u}(0). \quad (12)$$

This is the relation between overall force and displacement in the middle of the focal adhesion. For the Green's function, this displacement would diverge, because it only describes the far field.

The surface deformation created by a tangential traction force distributed in a Hertz-like manner over a circular area is known from contact mechanics [3]. Employing Cartesian coordinates centered around the center of the circular adhesion, and the abbreviation $r = \sqrt{x^2 + y^2}$ the surface traction profile is given by:

$$\boldsymbol{\tau}(x, y) = \begin{cases} \frac{3\mathbf{F}}{2\pi a^3} \sqrt{a^2 - r^2} & r < a \\ 0 & r \geq a \end{cases} \quad (13)$$

A linear cross-section of this deformation profile is shown in Fig. 1. The corresponding deformation field is given by:

$$u_x(x, y) = \frac{3(1 + \nu)}{8Ea^3} ((W_1 + W_2)F_x + W_3F_y) \quad (14)$$

$$u_y(x, y) = \frac{3(1 + \nu)}{8Ea^3} (W_3F_x + (W_1 + W_4)F_y) \quad (15)$$

The functions W_1 to W_4 have the following form in the inner region where $r < a$:

$$W_1 = \frac{1}{4}4(2 - \nu)a^2 \quad (16)$$

$$W_2 = -\frac{1}{4}((4 - 3\nu)x^2 + (4 - \nu)y^2) \quad (17)$$

$$W_3 = \frac{1}{4}2\nu xy \quad (18)$$

$$W_4 = -\frac{1}{4}((4 - 3\nu)y^2 + (4 - \nu)x^2). \quad (19)$$

For the outer region where $r > a$ and $\xi_2 = a^2/r^2$ we have:

$$W_1 = \frac{2 - \nu}{\pi} \left((2a^2 - r^2) \arcsin \frac{a}{r} + ar \sqrt{1 - \frac{a^2}{r^2}} \right) \quad (20)$$

$$W_2 = \frac{\nu}{2\pi} \left(r^2 \arcsin \frac{a}{r} + (2a^2 - r^2) \frac{a}{r} \sqrt{1 - \frac{a^2}{r^2}} \right) \frac{x^2 - y^2}{r^2} \quad (21)$$

$$W_3 = \frac{1}{\pi} \left(r^2 \arcsin \frac{a}{r} + (2a^2 - r^2) \frac{a}{r} \sqrt{1 - \frac{a^2}{r^2}} \right) xy \quad (22)$$

$$W_4 = \frac{\nu}{2\pi} \left(r^2 \arcsin \frac{a}{r} + (2a^2 - r^2) \frac{a}{r} \sqrt{1 - \frac{a^2}{r^2}} \right) \frac{y^2 - x^2}{r^2}. \quad (23)$$

The shape of this deformation is also shown in Fig. 1.

In the limit $r \rightarrow 0$, we find, that:

$$\mathbf{F} = \frac{8aE}{3(1 + \nu)(2 - \nu)} \mathbf{u}(0). \quad (24)$$

Compared with Eq. (12), we see that both scale linear in Young's modulus and patch size. Also the contribution related to the Poisson ratio is equivalent. They differ only in the constant prefactor.

In general, the deformation fields in both cases share many similarities. In both cases the absolute value of the deformation field $u_{abs} = \sqrt{u_x^2 + u_y^2}$ takes its maximal value at the center of the deformation. In addition the isolines of the u_{abs} field enclose simple connected regions always containing the center of the coordinate system. We define A_h to be the area where $u_{abs}(x, y) > hu_{abs}(0, 0)$. While the deformation field $\mathbf{u}$ is dependent on five parameters E , ν , a , F_x and F_y , only two of them a and ν will affect A_h . Because of the way we can choose our unit scale, it can be easily seen, that a contributes quadratically ($A_h \propto a^2$). A numerical analysis (Fig. 1) reveals that the relation to ν can be estimated using a linear function. Therefore, the expression $A_h = (y_h + m_h \nu) a^2$ describes the relationship between A_h , a and ν . The constants y_h and m_h can be determined numerically by simulating the situation for an arbitrary choice of the five parameters mentioned above. An estimation of the total force $\mathbf{F}$ of

an Hertz-like or constant-traction contact based on the deformation field can be found using Eq. (12) or (24), by finding the deformation at the a center of the contact. The contact radius a can be calculated from the isoline-enclosed area A_h for some value h .

The overall algorithm to determine forces $\mathbf{F}_i$ within each adhesion now contains the following steps done individually for each adhesion search area i :

1. Interpolate the deformation field onto a regular spaced square grid for each time step.
2. Calculate the absolute value $u_a b s$ of the deformation field for each time step.
3. Locate the center of the adhesion by making use of the fact that $u_a b s$ should reach its maximum in this location for each time step.
4. A common issue in the above estimation is the fact that the adhesion in adjacent search areas might cause the center of the current adhesion not to correspond to the global maximum of $u_a b s$ within its search area, in which case the largest value for $u_a b s$ can be found right next to the adhesion search area boundary. In these cases, we rely on an interpolation from the other time-steps to select the presumed location for the area estimate.
5. Now that we have determined the center of the adhesion for each time step and the radius a , we can determine the deformation $\mathbf{u}$ in the adhesion center.
6. Calculate the area A_h within the adhesion search area where $u_a b s$ lies within $1 - h = 5\%, 10\%, 20\%, 30\%$ of its maximal value for each time step.
7. For each time step and each threshold value an estimate for the adhesion radius a can now be determined using the above mentioned area formula $a = \sqrt{A_h / (y_h + m_h \nu)}$ using the predetermined values for y_h and m_h . In general, all of this estimates should yield a similar value, as the radius of the adhesion is expected not to change during the procedure.
8. The final estimate for the adhesion radius can now be found by finding the mean of the estimates determined in the previous step. We explicitly omit those time steps from the calculation, where we had to use the interpolation from the other time-steps in step 4, as the estimates in these cases are particularly unreliable.
9. Now that we have determined the deformation $\mathbf{u}$ in the center of each adhesion for each time step and the radius a , we can determine the corresponding force $\mathbf{F}_i$ using Eq. (12) or (24).

In order to determine whether adhesion sites can be better described by Hertz-like or the constant-traction profiles, we compare the statistic variance

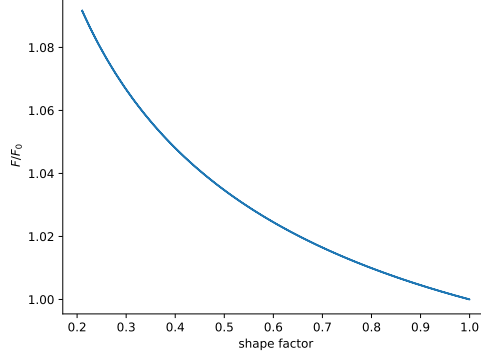


Figure 2: Ratio between the predicted force for an elliptical contact and a circular contact of equal contact area F/F_0 for $\nu = 0.5$ using Eq. (26). The horizontal axes describes the shape factor given as defined by [4].

between the radius estimates derived from different search area estimates in step 7. As can see (Fig 1. G and H) both approaches give a similarly consistent image in this regard. As the constant-force approach gives a slightly better estimate, we chose the constant traction estimate for our experimental analysis.

These two approaches assume a circular shape of the adhesion area, while many recent studies have suggested a more elliptic shape [4]. In case of a Hertz like contact, the surface traction profile can be modeled, if we assume that both the direction of the force, as well as one of the semi-axes are parallel to the x-axes:

$$\boldsymbol{\tau}(x, y) = \begin{cases} \frac{3F_x \mathbf{e}_x}{2\pi ab} \sqrt{1 - \frac{x^2}{a^2} - \frac{y^2}{b^2}} & \frac{x^2}{a^2} + \frac{y^2}{b^2} < 1 \\ 0 & \frac{x^2}{a^2} + \frac{y^2}{b^2} \geq 1 \end{cases} \quad (25)$$

The deformation field for force central displacement relation at the center is given by [5]:

$$\mathbf{F} = \frac{8\sqrt{ab}E}{3N(\nu, a/b)(2-\nu)(1+\nu)} \mathbf{u}(0) . \quad (26)$$

The function $N(\nu, a/b)$ is given by

$$N\left(\nu, \frac{a}{b}\right) = \begin{cases} \frac{4}{\pi(2-\nu)} \sqrt{\frac{a}{b}} \left(K_0(m_a) - \nu \frac{K_0(m_a) - E_0(m_a)}{m_a} \right) & a < b \\ 1 & a = b \\ \frac{4}{\pi(2-\nu)} \sqrt{\frac{b}{a}} \left((1-\nu)K_0(m_b) + \nu \frac{K_0(m_b) - E_0(m_b)}{m_b} \right) & a > b \end{cases} \quad (27)$$

with the definition $m_a = 1 - a^2/b^2$ and $m_b = 1 - b^2/a^2$. Its inverse $1/N$ describes the ratio between the force predicted using Eq. (26) and the one predicted using Eq. (24) for a circular adhesion of equal area. In Fig. 2 $1/N$ is plotted against the

shape factor $\frac{\pi^2}{4} \frac{b}{a} \frac{1}{E_0(m_a)^2}$. Assuming a shape factor of 0.5 as observed by [4], we see that the force increase is only around 4% which is likely below the accuracy of the prediction.

Calculation of force moments

The force monopole is the net directed force on the substrate and defined by

$$\mathbf{F} = \int \boldsymbol{\tau} d^2x . \quad (28)$$

Knowing the adhesion patches, this means that we can find the total force monopole vector by simply summing up the force contributions of all adhesions:

$$\mathbf{F} = \sum_k \mathbf{F}_k . \quad (29)$$

Due to momentum conservation, the force monopole should be equivalent to the force transmitted by the cantilever into the cell.

We also define the first order moment matrix $\mathbf{M}$ as [6]

$$M_{ij} = \int x_i \tau_j d^2x . \quad (30)$$

The coordinate frame for this integral is chosen with respect to the center of force of the system, which is given by

$$\mathbf{x}_{\text{CF}} = \left(\int |\boldsymbol{\tau}| d^2x \right)^{-1} \int |\boldsymbol{\tau}| \mathbf{x} d^2x \quad (31)$$

which can be calculated in any coordinate frame.

Inserting the traction profile for patches (Eq. 13 or Eq. 1) yields:

$$\mathbf{x}_{\text{CF}} = \left(\sum_k |\mathbf{F}_k| d^2x \right)^{-1} |\mathbf{F}_k| \mathbf{x}_k d^2x , \quad (32)$$

$$M_{ij} = \sum_k (\mathbf{x}_k)_i (\mathbf{F}_k)_j d^2x . \quad (33)$$

The diagonal components of the moment matrix describe the contractility of the system. The two off-diagonal components corresponds to a torque relative to the center of force [6]. We define the contractile momentum by

$$\mu = M_{11} + M_{22} . \quad (34)$$

This describes the net ability to dilate or contract the cell. The net torque is defined by

$$\mathcal{M} = M_{12} - M_{21} . \quad (35)$$

Both μ and $\mathcal{M}$ are independent of the orientation of the coordinate axes and are independent of each other.

Without needle pulling, angular momentum conservation dictates that the net torque is zero and the moment matrix symmetric. In this case, one can find

an orientation of the axis such that $\mathbf{M}$ is diagonal and the eigenvalues can be used to find the directed and isotropic contractile moment of the system. To also consider the case of needle pulling, we define a slightly modified version of the moment matrix, where we removed the torque contribution:

$$M_{ij}^{\parallel} = \int x_i x_j \frac{\boldsymbol{\tau} \cdot \mathbf{x}}{\mathbf{x}^2} d^2 x . \quad (36)$$

If we again insert the definition of the patches, we obtain:

$$M_{ij}^{\parallel} = \sum_k (\mathbf{x}_k)_i (\mathbf{x}_k)_j \frac{\mathbf{F}_k \cdot \mathbf{x}_k}{\mathbf{x}_k^2} . \quad (37)$$

This matrix is symmetric and thus an orthogonal eigendecomposition can be found. The two eigenvalues describe the dipole moments and the eigenvector corresponding to the major dipole describes the main contractile axis. This can be proven by comparing the trace of $\mathbf{M}^{\parallel}$ to the contractile momentum μ , which both yield the same value.

Young's modulus of PDMS-pillars used for microneedle calibration

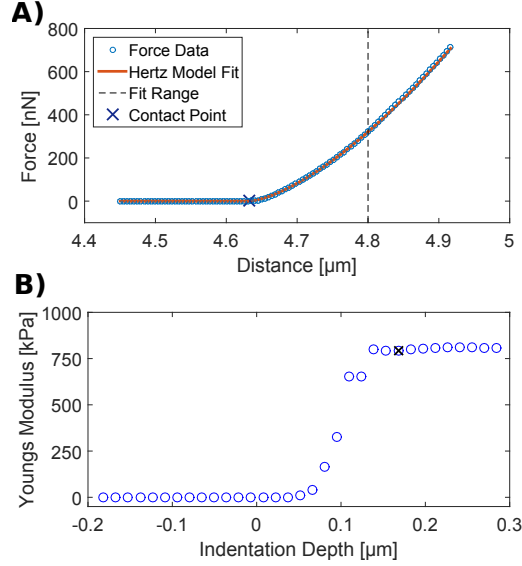


Figure 3: The Young's modulus of the PDMS pillars employed for the calibration of the microneedle was measured using an AFM indentation based method published by Huth et al. [7]. A silica bead with 21 μm diameter was glued to an AFM cantilever and was subsequently used to indent the PDMS sample with a setpoint force of 700 nN at 1 $\mu\text{m/s}$. **A** shows an exemplary indentation curve with a Hertz model fit. Our method employs an algorithm that calculates the Young's modulus for different indentation depths. The resulting curve is presented in **B**. The Young's modulus is determined by finding a plateau in this curve and finding the value with lowest fitting residuals. This value is marked in **B** with a black cross. The sample was indented 16 times at each of 32 different positions resulting in a mean value of 801.49 ± 32.91 kPa.

Young's modulus of the polyacrylamide sample

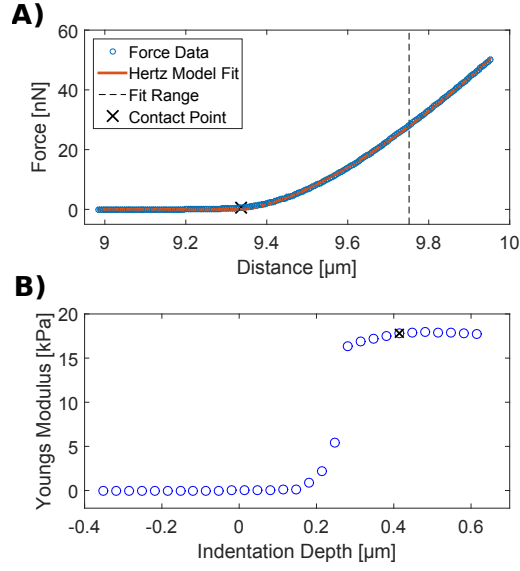


Figure 4: The Young's modulus of a polyacrylamide (PAAm) sample that serves as a traction force microscopy substrate needs to be known in order to calculate traction forces from the displacement of the beads embedded in the sample. We employed the same method to measure the Young's modulus of our PAAm samples as for the PDMS pillars. Indentation curves were collected at a cantilever speed of 1 $\mu\text{m/s}$ and a setpoint force of 50 nN. **A** shows an exemplary indentation curve with a Hertz model fit, while **B** shows the resulting Young's modulus versus indentation depth. The sample was indented 16 times at each of 30 different positions resulting in a mean value of 16.49 ± 0.55 kPa.

Traction forces in perpendicular direction

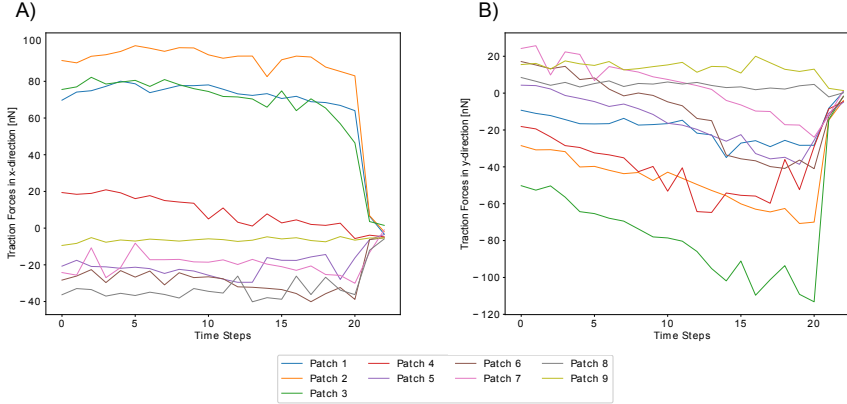


Figure 5: Traction force data from the cell presented in Fig. 2 of our main manuscript. **A** shows the x-components of the traction force vectors. It is very clear the these are not influenced by the shearing in y-direction, which is why we focus our discussion on traction forces in y direction. **B** shows the y-components of the traction forces for each adhesion patch. We decided to combine some neighbouring patches with similar behavior for better visualization. We combined patches 2,3 and 4 as well as 5,6 and 7.

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