Note 1 Stiffness tensor of a semi-affine material

The stiffness tensor is defined as the derivative of the nominal stress by the deformation gradient.

\[ K_{ijkl} = \frac{\partial N_{ij}}{\partial F_{kl}} \]

In a linear material, the stiffness tensor is independent of the deformation state of the material. Since our constitutive equation is non-linear, however, the stiffness tensor depends on the deformation. Differentiation of Eq. 3 (main text) gives

\[ K_{ijkl} = \frac{\partial N_{ij}}{\partial F_{kl}} = \left\langle e_{\Omega i} \cdot e_{\Omega j} \cdot \left( \frac{|F \cdot \vec{e}_{\Omega}| \cdot w''(|F \cdot \vec{e}_{\Omega}| - 1) - w'(|F \cdot \vec{e}_{\Omega}| - 1)}{|F \cdot \vec{e}_{\Omega}|^2} \right) \right\rangle_{\Omega}; \quad k \in \{x, y, z\}; \quad l \in \{x, y, z\} \]

This exact expression of the stiffness tensor is used further below for the traction force reconstruction in a non-linear material. For readers who are interested to know how this stiffness tensor is related to the more familiar description of mechanical properties in the framework of linear elastic theory, we provide the following explanation. In linear elastic theory, the stiffness of a material is fully defined by two parameters, for example the Young’s modulus \( Y \) and the Poisson’s ratio \( \nu \). To relate the 4 parameters of our constitutive equation to \( Y \) and \( \nu \), we approximate the stiffness tensor \( K \) for small strain \( (F \approx I) \). In this case, the fiber stretch \( \lambda \) is 0 for all solid angles \([\theta, \phi] = \Omega\), which yields the following simplifications:

\[ w(\lambda) = 0; \quad w'(\lambda) = 0; \quad w''(\lambda) = \kappa_0; \]

We can therefore rewrite the stiffness tensor

\[ K_{ijkl}(F = I) = \kappa_0 \cdot (e_{\Omega i} \cdot e_{\Omega j} \cdot e_{\Omega k} \cdot e_{\Omega l})_{\Omega} \]

The stiffness for extension, as measured in our extensional rheometer experiments, therefore can be expressed as a function of \( Y \) and \( \nu \) [ZienkiewiczV1 page 132]

\[ K_{zzzz}(F = I) = \frac{1}{4\pi} \cdot \kappa_0 \cdot \int_{\Omega} \cos(\theta)^4 d\Omega = \frac{\kappa_0}{5} = \frac{Y \cdot (1 - \nu)}{(1 + \nu)(1 - 2\nu)} \]
Similarly, the stiffness perpendicular to the stretch direction is given by

\[ K_{zzyy}(\mathbf{F} = \mathbf{I}) = \frac{1}{4\pi} \cdot \kappa_0 \cdot \int \cos(\theta)^2 \cdot \sin(\theta)^2 \cdot \sin(\phi)^2 \, d\Omega = \frac{\kappa_0}{15} = \frac{Y \cdot \nu}{(1 + \nu)(1 - 2\nu)} \]

Together, it follows that

\[ Y = \frac{\kappa_0}{6} \text{ and } \nu = 0.25 \] (3)

As described below, we experimentally confirm a Poisson’s ratio of \( \nu = 0.25 \) for a linear elastic biopolymer network such as Matrigel.

**Note 2  Hyperelastic finite element method**

The finite element method is used to numerically compute solutions to mechanical boundary value problems. In most applications, linear materials are considered. To solve boundary value problems imposed by a contracting cell embedded in a collagen biopolymer network, however, we need a fully hyperelastic finite element model. The geometry of the material, in this case the collagen gel, is discretized into multiple tetrahedral elements, which together fill the entire space of the material. Neighboring elements share one or more of their corner points. The displacement field inside the material is discretized to these corner points (the nodes) and linearly interpolated inside the tetrahedra. Because of this linear interpolation, the deformation gradient \( \mathbf{F} \) is constant over the volume of a tetrahedron. The deformation gradient is the linear map of the undeformed tetrahedron \( T \) onto the deformed tetrahedron \( T' \) [Ogden page 84].
Figure S 1: (A) A tetrahedron $T^\ast = [\vec{r}_a, \vec{r}_b, \vec{r}_c, \vec{r}_d]$ is deformed by displacements $\vec{u}_a, \vec{u}_b, \vec{u}_c$ and $\vec{u}_d$ at its corner points. The primitive tetrahedron $P$ has three of its edges aligned with the coordinate axes $\vec{e}_x, \vec{e}_y$ and $\vec{e}_z$. Linear maps (B and A) of $P$ onto $T$ as well as $T'$ are straightforward to compute. The linear map $F$ of $T$ onto $T'$ is then simply given by $A \cdot B^{-1}$. (B) Tetrahedral elements of the finite element model are filled with fiber elements, and the stresses at the faces of each tetrahedron are computed by averaging the individual forces of the intersecting fibers.

Next, we need to find the matrix $B$ that describes the linear map of the undeformed tetrahedron $T$ onto the primitive tetrahedron $P$ that is defined by corner points $\{(0, 0, 0) (1, 0, 0) (0, 1, 0) (0, 0, 1)\}$ (Fig. 1). $B$ is given by the matrix with the vectors $(\vec{r}_b - \vec{r}_a)$, $(\vec{r}_c - \vec{r}_a)$ and $(\vec{r}_d - \vec{r}_a)$ as column vectors. We also need the matrix $A$ that describes the linear map of $P$ onto the deformed tetrahedron $T'$. It is given by the matrix with the vectors $(\vec{r}_b - \vec{r}_a + \vec{u}_b - \vec{u}_a)$, $(\vec{r}_c - \vec{r}_a + \vec{u}_c - \vec{u}_a)$ and $(\vec{r}_d - \vec{r}_a + \vec{u}_d - \vec{u}_a)$ as column vectors. The linear map $F$ that maps $T$ to $T'$ is then given by $A \cdot B^{-1}$. Using the 4x3 helper tensor

$$X_{mk} = \begin{pmatrix} x & y & z \\ -1 & -1 & -1 \\ 1 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 1 \end{pmatrix}$$

and using Einstein notation, the deformation gradient matrix $F$ can be rewritten as a function of the nodal displacements $\vec{u}_m$:

$$A_{ij} = B_{ij} + u_{mi} \cdot X_{mj}; \ m \in \{a, b, c, d\}; \ i \in \{x, y, z\}; \ j \in \{x, y, z\}$$

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\[ F_{ij} = B_{ik} \cdot B_{kj}^{-1} + u_{mi} \cdot X_{mk} \cdot B_{kj}^{-1}; \quad k \in \{x,y,z\} \]

\[ = \delta_{ij} + u_{mi} \cdot X_{mk} \cdot B_{kj}^{-1} \phi_{mj} \]

where \( \phi \) is the shape tensor of the tetrahedron and \( \delta_{ij} \) is the Kronecker delta. The derivative of \( F \) with respect to the displacements \( \vec{u}_m \), which when multiplied with the stress tensor \( N \) gives the nodal forces \( \partial E_T \partial u_{ml} \) of the tetrahedron \( T \) (\( E_T \) denotes the strain energy of the tetrahedron \( T \)), is then given by

\[ \frac{\partial F_{ij}}{\partial u_{mk}} = \delta_{ik} \cdot X_{mn} \cdot B_{nj}^{-1} = \delta_{ik} \cdot \phi_{mj} \quad (4) \]

\[ \frac{\partial E_T}{\partial u_{ml}} = V_T \cdot \frac{\partial W_T}{\partial F_{ij}} \frac{\partial F_{ij}}{\partial u_{ml}} = V_T \cdot N_{Tij} \cdot \phi_{mj} \quad (5) \]

Next, the equilibrium condition (Cauchy’s law of motion) is formulated for a tetrahedral mesh. This is done by applying the principle of virtual work, which states that a deformation is a solution to a given boundary value problem if and only if the variation of the potential energy vanishes for all admissible small variations of the deformation \( \vec{\delta u} \). [Ogden page 312]

Below, we solve the boundary value problem of imposed displacements and tractions for a single tetrahedron \( T = [\vec{r}_a, \vec{r}_b, \vec{r}_c, \vec{r}_d] \). The faces of the tetrahedron are indexed in the following way:

\[ \alpha = [\vec{r}_b, \vec{r}_c, \vec{r}_d] \]
\[ \beta = [\vec{r}_c, \vec{r}_d, \vec{r}_a] \]
\[ \gamma = [\vec{r}_a, \vec{r}_b, \vec{r}_d] \]
\[ \delta = [\vec{r}_a, \vec{r}_b, \vec{r}_c] \]

\( A_{Tl} \) denotes the surface area of the face \( l \) of the tetrahedron \( T \). The surface traction at a given set of faces \( \{l\} \) is \( t_{Tl} \). Further, only a subset of the corner points of the tetrahedron \( \{d\} \subset \{m\} \) is free to move, and a constant body force \( \vec{b}_T \) (e.g. an external force from the cell or a magnetic bead) is acting on the tetrahedron with volume \( V_T \) and density \( \rho_0 \). We can write the variation of the potential energy as [Ogden page 308]

\[ \int_{V_T} \delta W \, dV - \int_{V_T} \rho_0 \vec{b}_T \cdot \delta \vec{u} \, dV - \int_{A_{Tl}} t_{Tl} \cdot \delta \vec{u} \, dA = 0 \quad (7) \]

Here, \( \delta \vec{u} \) are small variations to the displacements of the tetrahedron. The first term is the variation of the total strain energy inside the tetrahedron. The strain energy density is a function of the deformation gradient and is constant over the tetrahedron. We therefore rewrite the first term as a function of the variation of the displacements at the corner points \( \delta \vec{u}_m \).

\[ \int_{V_T} \delta W \, dV = V_T \cdot \frac{\partial W_T}{\partial F_{Tij}} \frac{\partial F_{Tij}}{\partial u_{mk}} \delta u_{mk} = \frac{\partial E_T}{\partial u_{mk}} \delta u_{mk} \quad (8) \]

In the second term of Eq. S 7, the variation of the displacements are averaged over the volume and multiplied with the body force. The second term can therefore be expressed as the arithmetic mean of the variation of the displacements at the 4 corner points.
Finally, in the third term of Eq. S 7, the variation of the displacements are averaged separately over the faces, which are subject to boundary tractions. The average surface displacements \( \bar{u}_{lk} \) are given by the arithmetic mean of the 3 displacements of the corner points framing the face of the tetrahedron. With the definition of the face index (Eq. S 6), we can derive the helper tensor

\[
h_{lm} = \frac{\partial \bar{u}_l}{\partial u_m} = \begin{bmatrix}
  a & b & c & d \\
  0 & \frac{1}{3} & \frac{1}{3} & \frac{1}{3} \\
  \frac{1}{3} & 0 & \frac{1}{3} & \frac{1}{3} \\
  \frac{1}{3} & \frac{1}{3} & 0 & \frac{1}{3} \\
  \frac{1}{3} & \frac{1}{3} & \frac{1}{3} & 0
\end{bmatrix}
\]

(10)

and write

\[
\int_{\Omega_T} \bar{u}_l \cdot \delta u \ dA = t_{Tlk} \cdot h_{lm} \cdot A_{Tl} \cdot \delta u_{mk}
\]

(11)

Summing up all three terms, we can rewrite the variation of the potential energy and then factor out the variation of the displacements at the corner points \( \delta u_m \):

\[
\frac{\partial E_T}{\partial u_{mk}} \delta u_{mk} - \rho_0 \cdot V_T \cdot b_T \cdot \frac{1}{4} \delta u_{mk} - t_{Tlk} \cdot h_{lm} \cdot A_{Tl} \cdot \delta u_{mk} = 0
\]

\[
= \delta u_{mk} \cdot \left( \frac{\partial E_T}{\partial u_{mk}} - \rho_0 \cdot V_T \cdot b_T \cdot \frac{1}{4} - t_{Tlk} \cdot h_{lm} \cdot A_{Tl} \right); \quad k \in \{x, y, z\}
\]

For the fixed nodes, the variation of the displacements are zero. Therefore, we sum only over the free corner of the tetrahedron, \( d \) instead of \( m \).

\[
\delta u_{dk} \cdot \left( \frac{\partial E_T}{\partial u_{dk}} - \rho_0 \cdot V_T \cdot b_T \cdot \frac{1}{4} - t_{Tlk} \cdot h_{ld} \cdot A_{Tl} \right) = 0; \quad d \in \{\text{free corner points of the tetrahedron}\}
\]

Since this equation has to hold for all possible variations of the displacements, the term in the brackets must be zero.

\[
\frac{\partial E_T}{\partial u_{dk}} - \rho_0 \cdot V_T \cdot b_T - t_{Tlk} \cdot h_{ld} \cdot A_{Tl} = 0
\]

(12)

In order to extend this equation to multiple connected tetrahedra, we sum over multiple tetrahedra. All arguments keep their meaning so that we can apply the summation directly to (Eq. S 12) using the helper tensor \( \theta \):

\[
\theta_{Tom} = \begin{cases} 
1, & \text{if the node } o \text{ is the corner point } m \text{ of the tetrahedron } T \\
0, & \text{else}
\end{cases}
\]

(13)

Again, we only sum over free nodes of the tetrahedral mesh:
We can rewrite this as a differential equation of the total strain energy of the body $E = \sum_T E_T$.

$$
(f_u)_{ok} = \frac{\partial E}{\partial u_{ok}} = \theta_{Tom} \cdot \left( \frac{\rho_0}{4} \cdot V_T \cdot b_{Tk} + t_{Tlk} \cdot h_{lm} \cdot A_{Tl} \right) = - (f_{\text{ext}})_{ok}
$$

with $f_{\text{ext}}$ denoting the external nodal forces. Therefore, the body is in equilibrium if the internal nodal forces $f_u$ counterbalance the external nodal forces, which can be calculated for any boundary value problem using Eq. S 14. In reverse, Eq. S 14 can be used to calculate the body forces, which describe external forces from a cell or a magnetic bead, given a set of nodal forces $f_u$ as retrieved from the unconstrained force reconstruction algorithm.

**Note 3 Discretization of the semi-affine elastic network model for tetrahedral meshes**

In our semi-affine elastic network description, the strain energy density is given by.

$$
W = \langle w(|\mathbf{F} \cdot \mathbf{e}_r(\Omega)| - 1) \rangle_{\Omega}
$$

To make it numerically accessible, the averaging over the solid angle $\Omega$ is substituted by averaging over a finite set of $N_b$ angles $\Omega_b$ that are isotropically distributed. We use the notation:

$$
\mathbf{s}_b = \mathbf{e}_r(\Omega_b)
$$

$$
\mathbf{s}_b' = \mathbf{F}_T \cdot \mathbf{s}_b
$$

The integration over the material volume is substituted by a summation over the volumes of tetrahedra $T$ that fill the space of the body (see previous section). With that, the strain energy is:

$$
E_T = \frac{1}{N_b} \sum_b w \left( |\mathbf{s}_b'| - 1 \right) \cdot V_T
$$

$$
E = \sum_T E_T
$$

$E_T$ is the energy stored inside the tetrahedron $T$. $V_T$ is the volume of the tetrahedron $T$. The nodal forces $f_u$ (Eq. S 14) are defined as the derivatives of the total energy with respect to the nodal displacements $\mathbf{u}_t$. In the following, we use the helper tensor $\theta$ (Eq. S 13).

$$
(f_u)_{ot} = \frac{\partial E}{\partial u_{ot}} = \sum_T \sum_m \theta_{Tom} \cdot \frac{\partial E_T}{\partial u_{ml}} ; T \in \{\text{tetrahedra}\} ; o \in \{\text{nodes}\} ; m \in \{a,b,c,d\} ; l \in \{x,y,z\}
$$

Here, $\frac{\partial E_T}{\partial u_{ml}}$ are the nodal forces of the tetrahedron $T$. 

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Using (Eq. 5.4) we obtain:

\[
\frac{\partial E_T}{\partial u_{ml}} = \frac{1}{N_b} \cdot V_T \cdot \sum_b \frac{\partial w(|s_b^j| - 1)}{\partial |s_b^j|} \cdot \frac{\partial |s_b^j|}{\partial F_{ij}} \frac{\partial F_{ij}}{\partial u_{ml}}
\]

Finally, the stiffness of the tetrahedral mesh \( K_u \) can be derived.

\[
(K_u)^{olpi} = \frac{\partial^2 E}{\partial u_{ol} \partial u_{pi}} = \sum_T \sum_r \sum_m \theta_{T_{om}} \cdot \theta_{T_{pr}} \cdot \frac{\partial^2 E_T}{\partial u_{ml} \partial u_{ri}} : r \in \{a, b, c, d\}; p \in \{\text{nodes}\}; i \in \{x, y, z\} \quad (17)
\]

\[
\frac{\partial^2 E_T}{\partial u_{ml} \partial u_{ri}} = \frac{\partial}{\partial u_{ri}} \left( \frac{1}{N_b} \sum_b w'(|s_b^j| - 1) \cdot \frac{s_b^i}{|s_b^j|} \cdot \Phi_{mj} \right) = 1 \cdot \sum_b \frac{w'(|s_b^j| - 1)}{|s_b^j|} \cdot \frac{\partial |s_b^j|}{\partial u_{ri}} \cdot \Phi_{mj} + \Phi_{mj} \cdot \frac{\partial}{\partial u_{ri}} \left( \frac{1}{N_b} \sum_b \left( \frac{w'}{|s_b^j| - 1} \right) \cdot \frac{s_b^i}{|s_b^j|} \right)
\]

This can be reexpressed by the stiffness tensor \( K \)

\[
\frac{\partial^2 E_T}{\partial u_{ml} \partial u_{ri}} = \sum_{j,k} V_T \cdot K_{T_{jki}} \cdot \Phi_{mj} \cdot \Phi_{rk}
\]

**Note 4 Model fit to bulk rheology**

To extract the four material parameters \( \kappa_0, d_0, \lambda_s \) and \( d_s \) from macrorheological measurements, we compute the response predicted by our constitutive equation and minimize the mismatch between data and prediction by varying the parameter values. Below, we describe how the model predictions are calculated for three different macrorheological experiments.

**Note 4.1 Shear rheometer**

The shear rheometer applies a simple shear deformation \( \gamma \) of the following form to the gel:

\[
F(\gamma) = \begin{pmatrix}
1 & \gamma & 0 \\
0 & 1 & 0 \\
0 & 0 & 1
\end{pmatrix}
\]

The resulting stress is then given by the partial derivative of the strain energy density with respect to the engineering shear strain \( \gamma \), which we compute numerically.
\[ \sigma(\gamma) = \frac{dW(F(\gamma))}{d\gamma} \] (18)

**Note 4.2 Extensional rheometer**

The extensional rheometer applies a uniaxial strain \( \epsilon \) of the following form to the gel:

\[
F(\epsilon) = \begin{pmatrix}
\epsilon & 0 & 0 \\
0 & 1 & 0 \\
0 & 0 & 1
\end{pmatrix}
\]

The resulting stress is then given by the partial derivative of the strain energy density with respect to the strain \( \epsilon \), which we compute numerically.

\[ \sigma(\epsilon) = \frac{dW(F(\epsilon))}{d\epsilon} \] (19)

**Note 4.3 Uniaxial stretch**

In this experiment, the deformation of the gel is described by two parameters. With the stretching device, we impose a horizontal strain \( \lambda_h \) and then measure the vertical strain \( \lambda_v \). The deformation gradient is given by

\[
F(\lambda_h, \lambda_v) = \begin{pmatrix}
\lambda_h & 0 & 0 \\
0 & 1 & 0 \\
0 & 0 & \lambda_v
\end{pmatrix}
\]

\[ \Rightarrow W(F(\lambda_h, \lambda_v)) = W(\lambda_h, \lambda_v) \]

To compute \( \lambda_v \), we minimize the strain energy density with respect to \( \lambda_h \) numerically.

\[ \lambda_v(\lambda_h) = \text{argmin}(W(\lambda_h, \lambda_v)) \] (20)

**Note 5 Concentration dependence of material parameters for collagen gels**

We measure the strain-dependent Young’s modulus as well as the vertical contraction as a function of the horizontal stretch for three different collagen concentrations (0.6, 1.2, and 2.4 mg/ml). By fitting our material model, we find that the Young’s modulus increases with the concentration while all other parameters remain unchanged. This is in agreement with the notion that only the density of fibers increases with the collagen concentration, but that the internal mechanics of individual fibers does not change.

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Linear stiffness</th>
<th>Linear range</th>
<th>Strain stiffening coefficient</th>
<th>Buckling parameter</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.6 mg/ml</td>
<td>447 Pa</td>
<td>0.0075</td>
<td>0.033</td>
<td>0.0008</td>
</tr>
<tr>
<td>1.2 mg/ml</td>
<td>1645 Pa</td>
<td>0.0075</td>
<td>0.033</td>
<td>0.0008</td>
</tr>
<tr>
<td>2.4 mg/ml</td>
<td>5208 Pa</td>
<td>0.0075</td>
<td>0.033</td>
<td>0.0008</td>
</tr>
</tbody>
</table>
Figure S 2: Mechanical properties of collagen gels with different concentrations. (Left) Material stress as a function of uniaxial strain measured in an extensional rheometer for three different collagen concentrations (solid lines). With increasing concentration, the gel becomes stiffer. Blue lines indicates the semi-affine model fit to the data. (Right) Vertical contraction as a function of the horizontal stretch measured for three different collagen concentrations (solid lines) and model fit (dashed line). The collagen gels with different collagen concentrations do not show strong differences in their vertical to horizontal contraction ratio. Vertical contraction is not strongly affected by the collagen concentration. For principal strains (vertical contractions) above 40%, data and fit deviate due to batch dependent variations of the buckling parameter.

Note 6  Material parameters of a fibrin gel

In agreement with published data, from extensional rheometer and uniaxial stretch measurements, we find that fibrin shows no strain stiffening at small strain (< 50%) but an abnormal Poisson’s ratio [Brown 2009]. In our model, the strain stiffening depends on the response of the fibers under stretch, whereas the abnormal Poisson’s ratio depends on the response of the fibers under compression. Hence, strain stiffening and vertical contraction under stretch are decoupled both in our model and in the data. During polymerization, fibrin gels form a dense but thin (< 1 µm) scalelike skin at the surface. The mechanical stiffness of the skin layer is considerably higher than that of the fibrin bulk. Therefore, we were unable to verify our model under point-like forces with a magnetic tweezer.

<table>
<thead>
<tr>
<th>4.0 mg/ml</th>
<th>Linear stiffness</th>
<th>Linear range</th>
<th>Strain stiffening coefficient</th>
<th>Buckling parameter</th>
</tr>
</thead>
<tbody>
<tr>
<td>2091 Pa</td>
<td>∞</td>
<td>∞</td>
<td>∞</td>
<td>0.002</td>
</tr>
</tbody>
</table>
Figure S 3: Fibrin gels show vertical contraction due to fiber buckling but no strain stiffening for strains up to 50%. (Left) Material stress as a function of uniaxial strain measured in an extensional rheometer for a fibrin gel. We find a linear response for strains up to 50%. (Right) Vertical contraction as a function of the horizontal stretch measured for a fibrin gel (solid lines) and the model fit (dashed line).

Note 7  Material parameters of Matrigel

For Matrigel (10 mg/ml, BD Bioscience, polymerized at 37 °C for 1 h), we find a constant strain-independent Young’s modulus of 394 Pa and a constant vertical to horizontal contraction ratio of 0.34, which exactly matches the prediction for a material in which the fibers are linear in compression and extension and therefore do not buckle. In this case the Poisson’s ratio is 0.25 (Eq. S 3). The semi-affine elastic network model predicts that the displacement field around a point-like force is also symmetric, in contrast to a material that shows buckling (Fig. 3 F+H, main text). This is in agreement with measurements where we record the displacement field around a magnetic bead that is laterally pulled with a force of 20 nN.

<table>
<thead>
<tr>
<th></th>
<th>Linear stiffness</th>
<th>Linear range</th>
<th>Strain stiffening coefficient</th>
<th>Buckling parameter</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 mg/ml</td>
<td>2364 Pa</td>
<td>∞</td>
<td>∞</td>
<td>∞</td>
</tr>
</tbody>
</table>
Figure S 4: Matrigel shows neither fiber buckling nor strain stiffening. (A) Material stress as a function of uniaxial strain measured in an extensional rheometer for Matrigel. We find a linear response for strains up to 50% (B) Vertical contraction as a function of the horizontal stretch measured for Matrigel (green) and model fit (blue). Matrigel shows a horizontal contraction ratio of 0.34, in agreement with the model prediction for fibers that do not buckle under compression. (C) The matrix displacement field around a magnetic bead to which we applied a force of 20 nN. (D) Model predictions for the displacement field agree with the measurements.

Note 8 Detailed influence of material parameters on bulk rheology

To investigate the influence of the material parameters ($\kappa_0$, $d_0$, $d_s$ and $\lambda_s$) on different non-linear effects, we analyze how the predicted macrorheological behavior changes when we independently vary the four material parameters. We find that the buckling coefficient $d_0$ has the largest effect on the vertical contraction under horizontal stretch (Fig. S 5 B). By contrast, the stiffening coefficient $d_s$ and especially the onset of fiber stiffening $\lambda_s$ have only a minor effect on the vertical contraction under horizontal stretch (Fig. S 5 A, C). The linear stiffness under shear is solely determined by the linear fiber stiffness $\kappa_0$ (Fig. S 5 E). The degree of strain stiffening is determined mostly by $d_s$ (Fig. S 5 D), and the onset of strain stiffening by $\lambda_s$ (Fig. S 5 F). Thus, the fit parameters have a clear physical meaning and show only a small covariance, which makes the fit to the data robust.
Figure S 5: Influence of model parameters on macrorheology. (A,B,C): Uniaxial stretch: Data in green, fit in blue, fit with altered parameters in pink and brown (D,E,F) Shear rheometer experiment: Data in green, fit in blue, fit with altered parameters in pink and brown.

Note 9  Simulations of collagen gel micromechanics

Figure S 6: Cut-open view of a tetrahedral mesh. (A) The whole mesh has a spherical shape (diameter: 800 µm). White box indicates the section shown in B. (B) Magnified view to the ellipsoidal cell inclusion with a length of 50 µm and a width of 30 µm. The mesh consist of 63510 tetrahedra with their density increasing towards the center to achieve a higher resolution at the location of the highest material stresses and deformations.
The Matlab package Distmesh [Persson] is used to generate tetrahedral meshes with full control over geometry as well as node density. The average tetrahedron quality of the used meshes is \(0.85\). The simulated collagen gel is a sphere with a diameter of at least \(800\, \mu\text{m}\) with sticky boundary conditions. In the center of the sphere is an ellipsoidal hole representing the cell. The contractility of the cells is modeled as a Neumann boundary condition (defining the force). We assume that the cell generates a constant stress \(N_{\text{cell}}\) throughout its body. From this, the tractions \(\vec{t}\) on the cell-gel-interface can be computed by multiplying the normal vector \(\vec{n}\) of the boundary tetrahedral faces with the stress tensor

\[
\vec{t} = \vec{n} \cdot N_{\text{cell}}
\]

For the magnetic tweezer experiments, we simulate the collagen gel response to a force acting on a spherical bead (diameter \(5\, \mu\text{m}\)). The bead is half embedded in a half space of collagen material so that the bead center is at the level of the collagen surface. The top surface of the collagen gel is modeled as a free boundary condition. The bead is able to rigidly move in the direction of the applied force but is not allowed to rotate.

The boundary value problem (force equilibrium) is solved by minimizing the total strain energy for the given force and displacement constraints. The strain energy density inside each tetrahedron depends only on its deformation gradient \(F_T\), which in turn depends on the displacements of its nodes. The total strain energy \(E\) of the material can be expressed as the sum over the strain energy density \(W(F_T)\) times the volume of every tetrahedron \(V_T\),

\[
E = \sum_T W(F_T) \cdot V_T
\]

We numerically compute the set of second order Taylor-series coefficients \(f_u\) (Eq. S 16) and \(K_u\) (Eq. S 17) of the total strain energy.

\[
E(u + \Delta u) = E_u + f_u \cdot \Delta u + \frac{1}{2} \Delta u \cdot K_u \cdot \Delta u
\]

Here, the dot product in \(f_u \cdot \Delta u\) stands for \(\sum_{o,j} (f_u)_o (\Delta u)_j\) with \(o \in \{\text{free nodes}\}\) and \(j \in \{x,y,z\}\). The equilibrium condition \(dE/du = -f_{\text{ext}}\) (Eq. S 14) then reduces to a set of linear equations:

\[
-f_{\text{ext}} = f_u + K_u \cdot \Delta u
\]

We use the conjugate gradient method to solve this equation. Because the material model is highly nonlinear, we iterate until convergence is reached. The combination of a semi-affine elastic network description with finite element analysis as described here is not limited to collagen gels but may also be used for other biopolymer networks.

We compare simulations of a collagen gel also to simulations of a linear material, for which we chose \(w''(\lambda)\) (Eq. 1, main text) to have a constant value such that both material models (linear and non-linear) show the same linear shear modulus of \(43\, \text{Pa}\). The resulting stiffness for the linear material is \(\kappa_0/2\) due to the absence of buckling. The linear material shows a constant and symmetric Poisson’s ratio of \(\nu = 0.25\), whereas the non-linear material shows an asymmetric and strain-dependent Poisson’s ratio that can exceed values of \(\nu = 4\).

**Note 9.1 Isotropic contraction and dilation of a spherical inclusion**

To compute the mechanical response of collagen gels to cellular forces, we consider dilating and contractile forces for spherical and elongated cells. First, we analyze the stress and strain distribution in the collagen network around a spherical hole. Dilating forces resemble the situation of a cell that attempts to push through a restricting network.
pore, or the situation of a growing cell cluster spheroid inside a collagen matrix. Contracting forces resemble the situation of a cell that spreads in a collagen matrix by adhesive and contractile forces. From the computed stress and strain distribution in the collagen network, we calculate the isotropic pressure inside the hole that is needed to invoke a certain change in hole diameter. In addition to modeling the non-linear behavior of collagen, we perform the same calculation also for a linear elastic material with the same linear shear modulus as collagen but without buckling or strain stiffening. As expected for a linear material, we find for both, contracting and dilating forces the same slope of the pressure versus hole diameter relationship. By contrast, for a non-linear collagen network, a much higher pressure is needed to dilate the hole by more than 10% compared to contracting the hole by 10% (Fig. S 7 C). This asymmetric behavior is attributable to the buckling vs. strain stiffening asymmetry of the fibers. Moreover, we find that during dilation, circumferential fibers close to the hole tauten and bear most of the stress (Fig. S 7 B), forming a stiff ring around the hole like a strait-jacket. This ring effectively shields the bulk of the collagen gel from mechanical stress and prevents the deformations to spread out. The stiffening of circumferential fibers also explains the strong steric hindrance for cell migration in collagen networks with small pore sizes [Friedl 2011, Wolf 2003, Wolf 2013, Zaman 2006]. By contrast, during contraction, the stresses and strains are conducted outwards by the radially stretched fibers and thereby spread over a large distance (Fig. S 7 A), resulting in a much softer response that is nearly indistinguishable from a linear material (Fig. S 7 C). As a possible consequence, the cell may not be able to spread and elongate, similar to the behavior that cells show on very soft 2-D substrates [Engler 2004]. This may explain why many cancer cell lines that are able to metastasize in vivo do not readily migrate in collagen gels [Mierke 2008].
Figure S 7: Collagen gels respond differently to dilating and contracting forces, depending on cell shape. (A, B) Fiber deformations around a contracting or dilating three-dimensional ellipsoidal hole. Calculations are performed on a FE grid. For better visualization, only a small number of randomly selected fibers are shown. Blue hues indicate compressed fibers, red hues indicate stretched fibers. Undeformed fibers are not visible. (A) During contraction, the strain is effectively conducted outwards and spreads over a large distance. (B) Under dilation, the strain is not conducted outwards due to fiber buckling. In addition, circumferential fibers tauten and stiffen. (C) The pressure needed to dilate or contract a spherical hole in a linear material (brown) and in a collagen gel (blue), both with the same linear shear modulus. The linear material shows a nearly symmetric response during contraction and dilation. By contrast, collagen gels show a pronounced asymmetric behavior. Strain stiffening is much stronger for dilating than for contracting forces.

Note 9.2 Polarized contraction of an ellipsoid inclusion

To explore how the stiffening response under contraction depends on cell shape, we consider a polarized contracting cell with an ellipsoidal shape, which resembles the common appearance of a migrating mesenchymal cell inside collagen. The cell generates a uniaxially contracting stress (or prestress) throughout its body, resulting in tractions on the cell-gel-interface. We then calculate the elastic response of the collagen to these tractions and the resulting relative cell length changes (cellular contraction). We find that cells with higher aspect ratio exhibit much smaller relative length changes for the same level of prestress (Fig. S 8 A). This equates to a considerably higher apparent stiffness of the material for the more polarized cells. The reason for the apparent stiffening of the material is that the material deformation at the cell poles, for a given cellular contraction, is larger in the case of an elongated cell [Zemel 2010]. Therefore, by shape polarization, the cells can effectively compensate for the small stiffness and weak stiffening response of collagen networks under contraction. Furthermore, in agreement with the literature, we find that matrix displacements around elongated cells in a non-linear collagen gel spread out over much larger distances, up to several hundred microns, compared to displacements in a linear material where they decay much more quickly (Fig. S 8 D) [Abhilash 2014].
Figure S 8: (A, B) Fiber deformations around a contracting or dilating three-dimensional ellipsoidal hole. Blue hues indicate compressed fibers, red hues indicate stretched fibers. Calculations are performed on a FE grid. For better visualization, only a small number of randomly selected fibers are shown. Undeformed fibers are not visible. Cells in A and B produce the same contractile stress of 51 Pa along their axis of polarization. For a more elongated cell (A, aspect ratio = 4.2), the material responds with less deformation than for a more spherical cell (B, aspect ratio = 1.7). (C) Contractile stress vs. contractile strain for different cell shapes in a linear material (brown) and in a collagen gel (blue) for different cell aspect ratios. (D) Calculated matrix displacement along the primary axis of an elongated cell (aspect ratio = 8.3) for a linear material (brown) and a collagen gel (blue) with the same linear shear modulus. Displacements in the collagen gel decay more slowly.

Note 9.3  Comparison of calculated and measured displacement fields around cells

We compare the calculated matrix displacement fields to measured matrix displacement fields around single cells in a collagen gel [Koch 2012]. We fit the position, orientation and contractile stress of an ellipsoidal inclusion so that the calculated matrix displacements best match the measured displacements around a highly invasive, elongated MDA-MB-231 breast carcinoma cell (Fig. S 9 E), and around a non-invasive, round but highly contractile A-431 vulva carcinoma cell (Fig. S 9 B). The aspect ratios of the contractile ellipsoids are chosen to match the measured cell contours. The measured displacement field around both cells are well reproduced with our constitutive equation (Fig. S 9 A,D), whereas the linear material model fails to reproduce the long-ranging deformations seen in the measurements (Fig. S 9 C,F). For the invasive, elongated breast carcinoma cell, we find that the measured data are best described by an ellipsoid with a uniaxial stress tensor with a magnitude of 289 Pa in the direction of the long cell axis. For the non-invasive, round vulva carcinoma cell, we find the best match with an isotropic stress.
tensor with a magnitude of only 12.7 Pa in every direction.

Figure S 9: Measured and calculated displacements in a collagen matrix around non-invasive A-431 vulva cancer cell (upper row), and invasive MDA-MB-231 breast cancer cell (lower row). Colors and line density indicate the magnitude of the matrix displacements. Box size is 200 µm. (B,E) Measured matrix displacements from published data [Koch 2012]. (A,D) Calculated displacements for a non-linear collagen gel around an ellipsoidal cell with dimensions taken from measurements [Koch 2012]. The contractile stress (12.7 Pa for A-431 cell, 289 Pa for MDA-MB-231 cell) was fitted to match the measured displacements. (C,F) Calculated displacements for a linear material fail to recapitulate the measurements.

Note 9.4 Mechanical anisotropy due to gel geometry

Collagen gels used for cell culture usually have a free top surface. To investigate the effects of this free surface on the local mechanical properties that cells feel at a certain depth in the gel, we compute the apparent stiffness of the gel as a function of depth, for a round cell (Fig. S 10 A: diameter 20 μm) as well as an ellipsoidal cell (dimensions 50 × 15 × 15 μm). The simulated cell culture dish has a width of 800 μm. The long axis of the cell is aligned with the direction of the contractile forces. From the simulations, we extract an apparent gel stiffness, which is defined as the amount of contractile stress that the cell generates, divided by the resulting relative contraction of its body. We find that below 200 μm, the free surface has no effect on the apparent stiffness. At a depth of 100 μm below the gel surface, however, we see that the anisotropy is highly dependent on the shape of the cell and its orientation relative to the gel surface. We find that the fixed rigid bottom of the dish has no effect on the cell-encountered stiffness (Fig. S 10). In the case of a linear material instead of a collagen gel, we find that the apparent stiffness is independent of the depth and the orientation of the cell.
Figure S 10: Apparent stiffness anisotropy resulting from a free surface of a collagen gel for a round cell (A: diameter 20 µm) as well as an ellipsoidal cell (B: dimensions 50 × 15 × 15 µm). Red lines show the response of a linear material, blue lines the response of a collagen gel. Dashed lines indicate that the cell is contracting horizontally, whereas solid lines indicates that the cell is contracting vertically. Below a depth of 200 µm in the gel, the free surface has no effect. The distance to the fixed surface has no effect on the apparent matrix stiffness for distances larger than 100 µm (depth less than 700 µm).

Note 10  Matrix creep response around a point-like force

To quantify the visco-elastic creep response of the collagen matrix, the displacement of 10 marker beads located near the magnetic bead are measured during application of a constant point-like force (data from Fig. 3 a, see main text). The displacements are normalized by their individual final displacement after 300 s. We find that after 3 s, the matrix displacements have reached 97% of the final displacements, indicating predominantly elastic material properties. As we measure the matrix deformations around cells 30 min after force relaxation, visco-elastic creep can be neglected.

Figure S 11: Displacement of marker beads around a point-like force as a function of the duration of force application. The displacements of the 10 individual beads are normalized by the maximum displacement at 300 s after force application.
Note 11  Test of unconstrained force reconstruction with known point-like force

Figure S 12: Experimental validation of the unconstrained force reconstruction method. (A) Measured displacement around a single point-like force on the surface of a collagen gel. (B) Regularized displacements for the same measurement. (C) Force density calculated from the regularized displacements shown in (B). (D) Reconstructed vs. applied force. Horizontal error bars indicate the error in the applied force due to variations in bead size. (E) The reconstructed force amplitude depends only weakly on the choice of the regularization parameter $\alpha$ for values above $\sim 0.3 \text{pN}^2/\mu\text{m}^2$. Individual measurements in gray, average from 13 measurements in red. Dashed line indicates the value of $\alpha = 0.003 \text{pN}^2/\mu\text{m}^2$ chosen for all subsequent calculations. (F) Reconstructed force density for each bead is projected onto the x-axis (gray lines). The average force density is shown in red. Zero corresponds to the position of the magnetic bead before force application. Reconstructed forces from a point-like source are systematically shifted to the left ($31 \mu\text{m}$ against the direction of force application) and are spread out with a standard deviation of $29 \mu\text{m}$.

To test our method of unconstrained force reconstruction and to find the appropriate value for the regularization parameter $\alpha$, we apply a force of $20 \text{nN}$ to a magnetic bead and reconstruct the force from the matrix displacements measured around the bead. This time, we do not constrain the spatial distribution of the force to a point. We find that the algorithm reconstructs a force distribution with a Gaussian shape of $29 \mu\text{m}$ width (Fig. S 12 C) and a center of mass that is scattered with a standard deviation of $5 \mu\text{m}$ between individual measurements. However, the force center is systematically shifted by $31 \mu\text{m}$ in the opposite direction of the applied force (to the left in Fig. S 12 D). This blurring and shifting of the reconstructed force results from the finite size of the elements, measurement noise, numerical regularization, and material inhomogeneities that are not captured by the continuous material model. Nonetheless, the method correctly reconstructs the force magnitude with an error of less than 1% on average and a standard deviation of 30% between individual measurements (Fig. S 12 F). Because the optimal value for the regularization parameter $\alpha$ is unknown, we repeat this computation for multiple values of $\alpha$. We
find that the total force (vector sum of all forces around the bead) depends only little on \( \alpha \) for values below \( 0.3 \, \text{pN}^2/\text{\( \mu \text{m} \)}^2 \). Above this value, the force penalty becomes too high and the total force decays (Fig. S 12 E). Below a value of \( 0.0003 \, \text{pN}^2/\text{\( \mu \text{m} \)}^2 \), noise forces in the bulk of the gel volume begin to appear (Fig. S 16). We choose a value of \( 0.003 \, \text{pN}^2/\text{\( \mu \text{m} \)}^2 \) for all subsequent measurements.

**Note 12 Measurement of displacements from confocal reflection stacks**

The z-drive of confocal microscopes has a uncertainty in the z-position due to mechanical effects and thermal drift. Alignment of the two image stacks (recorded before (stack1) and after (stack2) cell force relaxation) is used to remove the z-dependent drift and to reduce the impact of z-drive imprecision. The alignment algorithm is based on a rigid registration method. It replaces every image of stack2 by a weighted average of images in stack2 that correlate best with the corresponding image of stack1. First, the cross-correlation between a given image \( \text{im} \) of stack2 with the corresponding image of stack1 is computed. Next, \( \text{im} \) is shifted by voxel-increments until its cross-correlation with stack1 reaches a maximum. Every voxel of \( \text{im} \) is then replaced by the average of the stack2 voxel with the highest cross-correlation and its 26 neighboring voxels, weighted by their respective cross-correlation coefficient. This procedure is repeated for all images of stack2. Apart from effectively removing stage drift and z-drive imprecision, this procedure results in a slight low-pass filtering of the images (Fig. S 13) that stabilizes the subsequent step of particle image velocimetry for computing the gel displacement field.

In order to quantify the accuracy of the displacements measured from confocal reflection stacks, we twice recorded a section of a collagen gel without cells. The true displacement field between both stacks is zero at every point. Therefore, non-zero displacements characterize the error of the method. For a section size of \( 12^3 \) voxels, each voxel with dimensions \( 0.72 \, \mu \text{m}^3 \), we find errors of \( \sigma \sim 0.06 \, \mu \text{m} \) in the \( \text{x, y and z-direction} \) (Fig S 14 A-C). This error depends only weakly on the distance of the confocal z-sections between \( 0.35 \, \mu \text{m} \) and \( 1.4 \, \mu \text{m} \) (\( \text{x-y voxel dimensions of } 0.72 \, \mu \text{m} \)) (Fig. S 14 D). The error is approximately constant for section sizes between \( 5^3 \) and \( 32^3 \) voxels (Fig. S 14 E).
Figure S 14: Error of displacement measurements. (A-C) Error distribution in x (A), y (B) and z (C) direction, measured from two collagen stacks that were recorded consecutively with no cells present. The accuracy of the measured displacements is nearly isotropic. (D) Error in z-direction vs. distance of the confocal z-sections (slice thickness). (E) Error in x/y-direction vs. section size.

Note 13 Analysis of cellular forces

From the unconstrained force reconstruction algorithm, the cellular force field is retrieved as a set nodal forces. As a first step to quantify the cellular forces, the epicenter of the force field \( \vec{a} \) is computed. A unique characteristic of the force epicenter is that most forces are pointing directly towards it. Therefore, the epicenter is the point where the norm of the cross-products of the nodal forces with the vectors from their respective nodes to that point is a minimum:

\[
Q = \sum_n \left| \vec{f}_n \times (\vec{r}_n - \vec{a}) \right|^2 \quad n \in \{ \text{nodes} \} 
\]

(23)

To compute the \( \vec{a} \) that minimizes \( Q \), we reexpress \( Q \) using the Binet–Cauchy identity [ButlerPC].

\[
Q = \sum_n \left| \vec{f}_n \times \vec{r}_n \right|^2 - 2 \cdot \left( \vec{f}_n \times \vec{r}_n \right) \cdot \left( \vec{f}_n \times \vec{a} \right) + \left| \vec{f}_n \times \vec{a} \right|^2
\]

\[
= \left| \vec{f}_n \right|^2 \left| \vec{r}_n \right|^2 - \left( \vec{f}_n \cdot \vec{r}_n \right)^2 - 2 \left( \left| \vec{f}_n \right|^2 \vec{r}_n \cdot \vec{a} - \left( \vec{f}_n \cdot \vec{r}_n \right) \left( \vec{f}_n \cdot \vec{a} \right) \right) + \left| \vec{f}_n \right|^2 \left| \vec{a} \right|^2 - \left( \vec{f}_n \cdot \vec{a} \right)^2
\]

In component notation using the Einstein summation convention, we see that \( Q \) is a quadratic function of the epicenter coordinates \( \vec{a} \).
\[
Q = \sum_n f_{ni} f_{nj} r_{nj} - f_{ni} f_{nj} r_{nj} - 2 \cdot (f_{ni} f_{nj} - f_{ni} f_{nj}) \cdot a_j \\
+ a_i \cdot (f_{nk} f_{nj} \delta_{ij} - f_{ni} f_{nj}) \cdot a_j \\
i, j, k \in \{x, y, z\}
\]
where \(\delta_{ij}\) is the Kronecker delta. Thus we write

\[
Q = \text{const} + \sum_n -2 \cdot (f_{ni} f_{nj} - f_{ni} f_{nj}) \cdot a_j \\
= \text{const} + \sum_n -2B_j a_j + a_i A_{ij} a_j
\]

where

\[
B_i = \sum_n f_{ni} f_{nj} r_{nj} - f_{ni} r_{nj} f_{nj}
\]

and

\[
A_{ij} = \sum_n f_{nk} f_{nj} \delta_{ij} - f_{ni} f_{nj}
\]

The minimum of \(Q\) can be found by setting its derivative with respect to \(a_i\) to zero (hence the constant term in \(Q\) (independent of \(a_i\)) is irrelevant). This gives

\[
\frac{\partial Q}{\partial a_i} = 0 = -2B_i + A_{ik} a_k + a_j A_{ji}
\]

As the matrix \(A\) is symmetric, it follows that \(A \cdot \vec{a} = \vec{B}\), and the coordinates of the force epicenter are therefore

\[
\vec{a} = A^{-1} \cdot \vec{B}
\]

We define the contractility of a cell as the sum of the forces that point towards the epicenter:

\[
C_{\text{tot}} = \sum_n \frac{\vec{f}_n \cdot (\vec{r}_n - \vec{a})}{|\vec{r}_n - \vec{a}|}
\]

To quantify the geometry of the cellular force field, we separate the contractile forces of every node into the force contributions from three principal components of an orthogonal coordinate system \(\{\vec{e}_{\text{max}}, \vec{e}_{\text{mid}}, \vec{e}_{\text{min}}\}\) that is aligned with the force field of the cell. The contractility can thus be separated into three components \(C_{\text{tot}} = C_{\text{max}} + C_{\text{mid}} + C_{\text{min}}\). \(\vec{e}_{\text{max}}\) is oriented such that the corresponding contractility \(C_{\text{max}}\) is highest. \(\vec{e}_{\text{max}}\) is found by testing a set of 7000 isotropically distributed unit vectors. In the same way, \(\vec{e}_{\text{min}}\) is found as the axis of least contractility. \(\vec{e}_{\text{mid}}\) is computed as \(\vec{e}_{\text{mid}} = \vec{e}_{\text{max}} \times \vec{e}_{\text{min}}\). The polarity \(P\) of the force field is given by

\[
P = C_{\text{max}} / C_{\text{tot}}
\]

The polarity therefore quantifies the fraction of the contractile force that is oriented in a single direction.

In order to calculate to what extent the cell is affected by the strain stiffening of the collagen matrix, we consider how much work the cell has to invest to deform the collagen by an infinitesimal extra amount \(\epsilon\). Because \(\epsilon\) is

\[
\text{Nature Methods: doi:10.1038/nmeth.3685}
\]
small, we use the Taylor series expansion of the total energy (Eq. S 21).

\[ E(\vec{u} + \epsilon \cdot \vec{u}) = E_u + \epsilon \cdot f_u \vec{u} + \frac{\epsilon^2}{2} \cdot u \cdot K_u \cdot \vec{u} \]

The apparent stiffness of the collagen gel “seen” by the cell is given by the second derivative of the total energy by \( \epsilon \).

\[ k_{cell} = \frac{\partial^2 E(\vec{u} + \epsilon \cdot \vec{u})}{\partial \epsilon^2} = u \cdot K_u \cdot \vec{u} \]

We compute \( k_{cell} \) for collagen where we either consider the full non-linear stress-strain relationship, or where we consider only the buckling behavior during compression but not the strain-stiffening behavior during extension (material parameter \( d_s \to \infty \)). The ratio of both stiffness values quantifies the additional stiffness that the cell encounters because of strain stiffening, and was estimated to be \( 3.2 \pm 0.5\% \) (mean \( \pm \) se) for \( n = 38 \) MDA-MB-231 breast carcinoma cells (see main text).

The principal stretch, principal stress and principal stiffness of the collagen matrix (see Fig. 5, main text) are calculated from the reconstructed deformation fields as follows. For every tetrahedron \( T \), the direction of the highest positive stretch (\( \vec{q} \)) is determined. The associated stress can then be calculated from the stress tensor inside the tetrahedron (Eq. 3, main text) as

\[ p_{\text{prin},T} = \sum_{i,j} N_{Tij} q_i q_j \]

The principal stiffness is computed as the derivative of the principal stress with respect to the deformation in the direction of the principal stretch, utilizing the stiffness tensor \( K \) (Eq. S 1)

\[ k_{\text{prin},T} = \sum_{i,j,k,l} K_{ijkl} q_i q_j q_k q_l \]

For an isotropic linear material, the principal stiffness is (Eq. S 2)

\[ k_{\text{prin}} = \frac{Y \cdot (1-\nu)}{(1+\nu)(1-2\nu)} \]

Where \( Y \) is the Young’s modulus and \( \nu \) is the Poisson’s ratio.

Note 14  Test of force reconstruction algorithm with synthetic data

We test the robustness of the unconstrained force reconstruction method using synthetic data, for which measurement noise and signal level (contractility of a cell) are precisely controlled. The calculated deformations around a polarized contracting ellipsoidal cell (dimensions 15 x 15 x 50 \( \mu m \)) as discussed in section 6.2 are used as a signal. The contractility of the cell is chosen to be \( 0 \) nN, \( 5 \) nN, \( 30 \) nN or \( 100 \) nN, respectively. The displacement field is interpolated onto a regular mesh (grid constant = 15 \( \mu m \)). To account for measurement error, Gaussian noise of varying amplitudes ranging from 0 – 500 nm is added to the displacements of each node. Fig. S 15 shows the reconstructed total contractility as a function of the regularization parameter \( \alpha \) for different true contractilities and different noise levels. The total reconstructed contractility is largely independent of \( \alpha \) for \( \alpha \)-values above \( 3 \cdot 10^{-4} \frac{\mu m^2}{pN} \) and below \( 10^{-3} \frac{\mu m^2}{pN} \). For higher values of \( \alpha \), the contractility approaches zero as cellular forces are excessively penalized. For intermediate \( \alpha \)-values, the reconstruction algorithm slightly underestimates the true contractility of the cell. In addition, higher noise levels lead to a larger underestimation of the true contractility, but even for the highest noise level of 500 nm, the error remains below 15 nN. For a noise level of 60 nm and
$\alpha$-value of $3 \cdot 10^{-3} \mu m^2/pN$ as in our measurements, the relative error is negligible. The underestimation of large contractilities in the absence of noise (10\% for a contractility of 100 nN) arises because the deformation field around the ellipsoidal cell is calculated from the known surface tractions, whereas the reconstruction algorithm assumes a continuous force field. As further validation of the algorithm we repeat this procedure also for different mesh sizes. We find no appreciable differences in the reconstructed contractility. For a true contractility of 30 nN and a noise level of 100 nm, the reconstructed contractility is 31.3 nN for a mesh size of 7.5 \mu m, 29.1 nN for a mesh size of 10 \mu m and 29.9 nN for a mesh size of 15 \mu m.

Figure S 15: (A) Cut-open view of the finite element mesh around a contractile cell. The cell is modeled as an ellipsoidal hole inside a continuous material. The cell generates traction forces (red arrows) at its poles with a total contractility of 30 nN. The matrix reacts to these forces through displacements (blue arrows). (B) The matrix displacements in (A) are interpolated onto a regular grid. (C) To account for measurement error, Gaussian noise (300 nm) is added to the displacements. (D) The cellular forces (red arrows) are reconstructed by unconstrained force reconstruction. The reconstructed total contractility is 28.7 nN. Scalebar is 50 \mu m.
As described in the main text, we find that the reconstructed forces are systematically shifted away from the point of force application, both for point-like applied forces as well as cellular forces. To test whether this is due to the unconstrained force reconstruction algorithm or due to the inhomogeneity of the material, which is not captured by our material model, we analyze this shift in simulated data. The material model for the simulation is the same as for the reconstruction and does not comprise material inhomogeneity. The contractility of the simulated cell is 30 nN. The simulated displacements are interpolated onto a regular mesh with a mesh size of 7.5 µm. Then 100 nm Gaussian noise is added to the displacements of each node before the unconstrained force reconstruction algorithm is used to reconstruct the cellular forces from these displacements. We integrate the resulting force density along the axes perpendicular to the cell orientation. In the resulting plot we see that the reconstructed force maxima remain close to the cell surface (Fig S 17). This points towards the neglected material inhomogeneity as the main reason for the systematic shift of the reconstructed forces.
As with all force microscopy methods, the sensitivity depends foremost on the resolution and accuracy of the measured displacement field. Fig S 18 shows the erroneously reconstructed contractility versus the displacement noise level using the concentration-dependent material parameters for collagen gels with concentrations of 0.6, 1.2 and 2.4 mg/ml. For the MDA-MB-231 carcinoma cells investigated in our study (contractility $\approx 40 \text{nN}$) and the noise in the displacements that we achieve with our confocal microscope ($60 \text{nm rms}$), the relative error is below 5%, and the detection limit for the total cellular contractilities is $\sim 2\text{nN}$. We also analyze the local error in the reconstructed force density as a function of the measurement noise for different collagen concentrations. We find that the reconstructed error remains below $10^{-5}\text{pN/\mu m}^3$. This is several orders of magnitude below typical values of $\sim 10^{-1}\text{pN/\mu m}^3$ that we measure for cells.

**Figure S 18:** Contractility background noise (left) and background in local force density (right) for different collagen concentrations ($0.6 \text{mg/ml in blue, 1.2 mg/ml in green and 2.4 mg/ml in red}$) versus noise level of measured displacements, in absence of true cellular forces.

**Note 15  Robustness of unconstrained force reconstruction tested on real data**

To test the robustness of the unconstrained force reconstruction method on a dataset of a contractile breast carcinoma cell, we delete the measured displacement information on a fraction of the nodes and let the algorithm reconstruct the contractile force. This is implemented by altering the $P$ matrix (see method description in the
that flags valid displacement entries. We find that up to 75% of the displacement information can be deleted without appreciable deviations of the reconstructed total contractility. When more than 75% of the displacement information is deleted, however, the algorithm can no longer separate signal from noise, and the reconstructed total contractility decays.

Figure S19: Total reconstructed contractility vs. fraction of nodes without displacement information.

Note 16 Computation time of unconstrained force reconstruction software depending on mesh density

The computation time of the algorithm depends on the grid constant of the finite element mesh. For values between 5 \( \mu m \) and 20 \( \mu m \) we find that the computation time scales linearly with the number of mesh points and therefore with the grid constant to the \(-3rd\) power. For a grid constant of 7.5 \( \mu m \) as used here, the total computation time is \( \sim 3 \) h running on a single Pentium E6500 CPU core. This computation time includes the reconstruction of the displacement field from confocal reflection data. On a more modern Intel core i5-3470 CPU the computation time is only 53 min running on a single core at a grid constant of 7.5 \( \mu m \).
<table>
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<th>Computation Time</th>
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<tr>
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<tr>
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<tr>
<td>20</td>
<td>12h</td>
</tr>
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<td>40</td>
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*N: Number of Mesh Points
g: Grid Constant

~ N^0
~ N^1 ~ 1/g^3
~ N^2

Figure S 20: Total computation time vs. grid constant of the finite element mesh. Data are shown in blue. The fit (red) is a second order polynomial in the number of mesh points (N). The green lines correspond to the 3 terms of the polynomial. In the relevant regime between 5 µm and 20 µm, the computation time scales approximately linearly with the number of mesh points.

**Note 17  Comparison of constrained and unconstrained reconstruction of cell forces**

In the main text, we compare constrained and unconstrained force reconstruction for the same data set of matrix displacements around a point-like force applied to the surface of a collagen gel. There we find that the constrained force reconstruction has a slightly higher accuracy in the localization of forces, whereas the unconstrained force reconstruction has a higher accuracy in the force magnitude. To compare force magnitude and localization accuracy of the unconstrained and constrained force reconstruction algorithm, when measuring cells, we record the matrix displacement field around 15 HT1080 cells stably expressing TagRFP inside a 1.2 mg/ml collagen gel. From the fluorescence stack, we extract the points of the finite element mesh with a distance to the cell of less than half a mesh size (3.75 µm). For these points, we set the force penalty A_{ii} to 0 and for all other points to 0.003 pN^2/µm^2, instead of letting the algorithm find the values of A_{ii} through the iterative approach described in the main text. Thereby we constrain the cellular forces to the cell surface. We confirm that the reconstructed cell forces only appear on the cell surface (Fig. S 21 E). The displacement field that we fit using the constrained algorithm, however, overestimates the measured matrix displacements or the displacements fitted with the unconstrained method, with maximum values that appear closer to the cell (Fig. S 21). The reconstructed contractilities, however, deviate not as strongly (69 ± 15nN for the constrained and 63 ± 13nN for the unconstrained case (mean±se of n=15 cells)) as the differences in the fitted displacement fields would suggest. We find also no significant differences for the reconstructed cell polarity (0.56 ± 0.11 for the constrained and 0.42 ± 0.07 for the unconstrained case (mean±se of n=15 cells)). We see this as confirmation that the unconstrained force reconstruction is not inferior to a constrained method for computing total contractility or force polarity.
Figure S 21: Constrained and unconstrained reconstruction of cellular forces. (A) Measured displacement field around HT1080 cell stably expressing TagRFP (shown in gray). (B) Displacement field fitted using unconstrained force reconstruction. (C) Force density obtained from unconstrained force reconstruction. (D) Displacement field fitted while constraining the forces to the cell surface. The maximum displacements are higher and appear closer to the cell when compared to (A) and (B). (E) Force density obtained from constrained force reconstruction. Cell forces only appear on the cell surface. The force density therefore has higher maximum values. Scalebar is 100 µm.

**Note 18 Influence of mesh size and choice of α-value on unconstrained force reconstruction**

For 3 different mesh sizes (5 µm, 7.5 µm and 10 µm), we calculate the force density around an MDA-MB-231 cell embedded in a 1.2 mg/ml collagen gel using the unconstrained force reconstruction method with a range of values for the regularization parameter α between $10^{-4}$ nN²/µm² and $1$ nN²/µm². For all mesh sizes, the contractility decreases with higher values of α. For an intermediate range of α-values, however, the contractility is nearly constant. We observe that both, this α-range and the corresponding contractility shift towards higher values with decreasing mesh size (Fig. S 22 A).

To select the appropriate α-value for the chosen mesh size, we analyze the reconstructed force field around the cell for different α-values. If α is chosen too low, noise forces appear everywhere; if α is too high, the cellular forces are smeared out also towards regions far outside of the cell. To quantify this effect, we plot the force density as a function of the distance to the cell surface and compute the first moment, which gives the average distance between the force vectors and the cell surface. For all three mesh sizes, the average force distance to the cell surface shows a minimum (Fig. S 22 B). This minimum corresponds to a reconstructed force field that neither is excessively smeared out nor excessively noisy, and thus represents an optimum. The optimum α shifts towards lower values with increasing mesh size. Fig S 22 C-E shows the reconstructed force field for three different mesh sizes and α-values as indicated by the dashed lines in Fig S 22 A+B.
Figure S 22: (A) Cell contractility as a function of the regularization parameter $\alpha$ for three different mesh sizes (5 $\mu$m in blue, 7.5 $\mu$m in green and 10 $\mu$m in red). In all cases, the contractility decreases with higher values of $\alpha$. For intermediate values of $\alpha$, the contractility is nearly constant. (B) Weighted average distance of the reconstructed force vectors to the cell surface as a function of the regularization parameter $\alpha$ for the same mesh sizes as shown in (A). In all cases, the distance shows a minimum for intermediate values of $\alpha$ that shifts towards lower $\alpha$-values for increasing mesh sizes. (C,D,E) Reconstructed force field for three different mesh sizes and $\alpha$-values as indicated by the dashed lines in (A) and (B).

Note 19  3-D force fields around MDA-MB-231 cells

In the following we present the individual force fields around all measured MDA-MB-231 breast carcinoma cells ($n=38$) in collagen gels with a collagen concentration of 1.2 mg/ml.
Figure S 23: 3-D force density plots around different MDA-MB-231 breast carcinoma cells. Arrow density and color corresponds to the local force magnitude. For colorbar, see Fig. S 24. Total displayed volume is a cubic box with edge length of 200 µm. The bottom face of the displayed box shows a brightfield z-projection of the cell.
Figure S 24: For caption see Fig. S 23
Note 20  Lagtime dependent cross-correlations of cell shape, motility and contractility

As described in the main text, we simultaneously measure the time course of contractility, cell elongation, migration persistence and migration activity (Fig. 6, main text). The following figure displays the full lag time depend cross-correlation matrix. The correlation functions were computed for every cell separately and then normalized by the variance over all cells and time-points. Therefore, the auto-correlation functions for zero lag can deviate from unity for individual cells, but not on average. The error values were calculated by bootstrapping.

Figure S 25: Lagtime dependent cross-correlation matrix of the aspect ratio, contractility, migratory activity, migratory persistence and protrusive activity. All x-axes display the lagtime in minutes. A star indicates that the correlation is significant ($p < 0.05$) for zero lag.
Note 21 Influence of collagen concentration on cell contractility

In the main text, we describe that the cells are less elongated in the stiffer and denser collagen gels. To test whether impeded cell elongation is the reason why the cells are not able to contract more strongly in stiffer gels, as cells plated on stiffer 2-D gels normally do [Trichet2012], we analyze the sub-population of cells that have an aspect ratio of 2.0 or higher. The aspect ratio of the cells was determined from brightfield projections of the cells. We find that the elongated cells grown in collagen gels with a concentration of 1.2 mg/ml indeed show a significantly increased contractility compared a sub-population of similarly elongated cells grown in collagen gels with a concentration of 0.6 mg/ml. For cells grown in collagen gels with a collagen concentration of 2.4 mg/ml, we are not able to resolve differences do the low number of cells that have a sufficiently elongated shape.

![Figure S 26: Cell contractility of elongated cells measured in collagen gels with different collagen concentration. Only cells with an aspect larger than 2.0 were included in this analysis.](image)

References

- [ButlerPC] Personal communication, 2005, Butler JP


