Supplemental Material

Mapping the creep compliance of living cells with scanning ion conductance microscopy reveals a subcellular correlation between stiffness and fluidity

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Numerical Model

Suppl. Fig. S-1 | Numerical model for quantification. (a) Finite element simulations for fluid flow and deformation of an elastic sample calculated for vertical pipette positions corresponding to 99% ion current (top panel, \( z_0 = 1.6 r_i \)) and to 98% ion current (bottom panel, \( z = 0.4 r_i \)). (b) Vertical pipette position at 99% ion current, \( z_0 \), and at 98% ion current, \( z \), as a function of sample compliance \( J \). \( \delta_0 \) denotes the difference between the two positions at zero sample compliance \( J = 0 \). (c) Relative sample deformation, defined as \( \delta = z_0 - z - \delta_0 \), as a function of \( J \) for different values of inner half cone angle \( \alpha \). The parameters for the shown FEM simulations are \( J = 1.5 p_0^{-1} \) (panel a), \( \alpha = 4^\circ \) (panels a and b), and ratio of outer to inner opening radius \( r_o/r_i = 1.5 \). The dashed red traces denote linear fits (b) and fits of Equation (5) (c).
Creep Compliance and Complex Modulus of Living Cells Follow a Power-Law Model

Suppl. Fig. S-2 | Creep compliance and complex modulus of a living cell and power-law model. (a) Creep compliance $J(t)$ with a time relative to $t_s$ (start of creep measurement) recorded on a living cell (same data as Figure 1c, bottom panel) shown on a log-log scale with fits of power law (Equation (3), red dashed trace), Maxwell $J(t) = E^{-1}(1 + t/\tau)$, gives $E = 2.9$ kPa and $\tau = 0.23$ s, red dotted trace), and Kelvin-Voigt $J(t) = E^{-1}(1 - e^{-t/\tau})$, gives $E = 2.3$ kPa and $\tau = 3.5$ ms, red dashed-dotted trace] models. Here, $E$, $\eta$, and $\tau = \eta/E$ denote modulus of elasticity, viscosity, and time constant, respectively. Interpreting the time constant in terms of a poroelastic material model gives poroelastic diffusion constants of typically $D_p = L^2/\tau \approx 10 \mu m^2 s^{-1}$ (using $L \approx r_i$ as characteristic length scale), consistent with AFM experiments.

(b) Complex modulus $E^*(\omega) = E'(\omega) + i E''(\omega)$ calculated by the modified Fourier transform of the creep compliance data (solid traces) and power-law model (red dashed-dotted trace, prediction from the fit in the time domain data).

Verification on a Silicone Polymer Sample

Suppl. Fig. S-3 | Verification on a silicone polymer sample. Map of (a) sample height, (b) stiffness, and (c) fluidity recorded on a CY52-276 polymer with the nominal mixing ratio of 1:1 (part A to part B). (d) Scatter plot of stiffness $E_0$ vs. fluidity $\beta$ and histograms of stiffness $E_0$ (right) and fluidity $\beta$ (top) with an indication of log-normal and normal distributions (black curves), respectively. As expected, the polymer is homogenous in stiffness and fluidity, within a narrow range of $\bar{E}_0 = 28 \pm 1$ kPa and $\bar{\beta} = 0.25 \pm 0.03$ (average ± standard deviation). No strong correlation between $E_0$ and $\beta$ was observed. For a mixing ratio of 6:5 (part A to part B) we measured averages $\bar{E}_0 = 10$ kPa and $\bar{\beta} = 0.4$, which is in very good agreement with AFM data on the same polymer with a similar mixing ratio. The applied pressure was $p_0 = 150$ kPa. Scale bars: 5 µm (a-c).
Correlation for Cell Population and for Pharmacological Treatment

Suppl. Fig. S-4 | Correlation between average stiffness and fluidity for the population of cells and for cells during pharmacological treatment. (a) Average stiffness $\bar{E}_0$ vs. average fluidity $\bar{\beta}$ for the population of cells ($N = 17$ cells) with fit of Equation (4) (red line). (b) Average stiffness $\bar{E}_0$ vs. average fluidity $\bar{\beta}$ for cells ($N = 5$ cells) before and 30 min after pharmacological treatment with 2 µM cytochalasin D with fit of Equation (4). (c) Average scaling parameters $j_0$ and $\tau_0$ and average correlation coefficient $r$ obtained from subcellular correlations (see e.g. Fig. 3), from the population of cells (see panel a), and from pharmacological treatment (see panel b). Plots show average values (markers) and data of individual cells (dots); error bars indicate estimated standard deviation. The light red areas represent standard error of the fit (a, b).

Table S1 | Average scaling parameters $j_0$ and $\tau_0$ and average correlation coefficient $r$ obtained from subcellular correlations (see e.g. Fig. 3), from the population of cells (see Suppl. Fig. S-4a), and from pharmacological treatment (see Suppl. Fig. S-4a), provided as average * (scaling parameters) or ± (correlation coefficient) standard error.

<table>
<thead>
<tr>
<th></th>
<th>Scaling parameters</th>
<th>Correlation coefficient</th>
<th>Number of cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$j_0$ (kPa$^{-1}$)</td>
<td>$\tau_0$ (µs)</td>
<td>$r$</td>
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<tr>
<td>Subcellular correlation</td>
<td>0.368 ± 1.1</td>
<td>13.2 ± 1.9</td>
<td>−0.65 ± 0.03</td>
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<td>Population of cells</td>
<td>0.371 ± 1.2</td>
<td>14.3 ± 6.0</td>
<td>−0.72 ± 0.18</td>
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<td>Pharmacological treatment</td>
<td>0.258 ± 1.3</td>
<td>6.01 ± 6.4</td>
<td>−0.91 ± 0.14</td>
</tr>
</tbody>
</table>
Suppl. Fig. S-5 | Stiffness and fluidity of a living cell during cytoskeleton disruption and recovery. Whole sequence of topography images (top row) and maps of stiffness $E_0$ (middle row) and fluidity $\beta$ (bottom row) of the living fibroblast cell from Figure 4 during addition and washout of 2 µM cytochalasin D. Scale bars: 20 µm. See also Supplementary Video S1 for an animation of this sequence.

References