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Supplemental information

Hypergravity affects cell traction forces of fibroblasts

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Beam Theory under Hypergravity Conditions

The deflection of the micropillar as an elastostatic process is described by the Bernoulli beam theory. Perpendicular acting forces to the beam axis with small angular changes are described by the Euler-Bernoulli assumption for narrow beams. The deflection of the beam $\delta := \omega(x)$ is calculated from the curvature of the bending line as a differential equation:

$$\omega''(x) = -\frac{M}{EI}. \tag{S1}$$

M is the bending moment, E the Young's modulus and I the second momentum of area:

$$I = \frac{\pi}{64} d^4 \tag{S2}$$

including the beam diameter, d. Taking the boundary conditions for a beam with clamping $\omega(0) = 0$ and $\omega'(0) = 0$, the lateral force acting on the pillar is described by

$$F = \frac{3EI}{h^3}\delta \tag{S3}$$

with h the pillar length.

Gravity causes additional forces acting on the beam (Fig. S1A): the gravitational force of the pillar, $F_{\mathrm{g,Pillar}}$, and of the cell, $F_{\mathrm{g,Cell}}$, both are calculated by the differential Eq. (S1) with the moment of $M = F_{\mathrm{g}} z$. As a superposition, for the bending of the pillar it results:

$$\delta = \frac{F_{\mathrm{L}} h^3}{3EI} + \frac{F_{\mathrm{g,Cell}} \delta h^2}{2EI} + \frac{F_{\mathrm{g,Pillar}} \delta h^2}{2EI} \tag{S4}$$

and the total acting force

$$F = \frac{3}{2} \frac{2EI - \left(F_{\mathrm{g,Cell}} + F_{\mathrm{g,Pillar}}\right) h^2}{h^3} \delta_{\mathrm{total}}. \tag{S5}$$

The gravitational forces are calculated by

$$F_{\mathrm{g,Cell}} = \gamma\, m_{\mathrm{partial-Cell}}\, g, \tag{S6}$$

$$F_{\mathrm{g,Pillar}} = \gamma\, m_{\mathrm{Pillar}}\, g. \tag{S7}$$

Here, γ is the gravitational level, g the gravitational acceleration, m_{Pillar} the mass of one pillar and $m_{\mathrm{partial-Cell}}$ the partial mass of the cell that acts on one pillar. This partial mass can be calculated by using the ratio of the volumes:

$$\frac{V_{\mathrm{partial-Cell}}}{V_{\mathrm{Cell}}} = \frac{A_{\mathrm{partial-Cell}}\; h_{\mathrm{Cell}}}{\frac{\pi}{4}\, d_{\mathrm{Cell}}^2\; h_{\mathrm{Cell}}} = \frac{m_{\mathrm{partial-Cell}}}{m_{\mathrm{Cell}}}. \tag{S8}$$

The height of the cell, h_{Cell}, with a cylindrical volume cancels out. Thus, the partial mass of the cell only depends on three cell values: the mass, m_{Cell}, the diameter, d_{Cell}, and the partial area, $A_{\mathrm{partial-Cell}}$. The partial area is the part of the cell that acts on one pillar and can be calculated with the geometry of a hexagonal structure (Fig. S1B):

$$A_{\mathrm{partial-Cell}} = \frac{\sqrt{3}}{2} \left(S + d_{\mathrm{Pillar}}\right)^2. \tag{S9}$$

Here, S is the rim-to-rim distance between two pillars and d_{Pillar} the diameter of the pillar.

The substitution of (S9) into (S8) yields the mass of the partial cell in (S6):

$$m_{\mathrm{partial-Cell}} = \frac{2\sqrt{3}}{\pi} \left(\frac{S + d_{\mathrm{Pillar}}}{d_{\mathrm{Cell}}}\right)^2 m_{\mathrm{Cell}}. \tag{S10}$$

The mass of the pillar in (S7) is given by

$$m_{\mathrm{Pillar}} = \rho_{\mathrm{Pillar}}\, V_{\mathrm{Pillar}}. \tag{S11}$$

Here, ρ_{Pillar} is the density of the pillar material and V_{Pillar} its cylindrical volume.

Background Analysis

Analyzed were the background deflections of the selected pillar regions outside the cell area as explained in the Materials and Methods - Image Analysis section. The mean deflection for each region is shown in Fig. S2A-C. Deflection of 10 nm is equal to 412 pN. A total of 931 background regions corresponding to the number of cells and 100,603 background pillars were analyzed.

We measured for Array6 the lowest background and Array4 the highest background with (37.3 ± 2.8) nm and (46 ± 6) nm, respectively. This results in a difference of 8.9 nm. In upright orientation, the difference between the lowest background of 1*g* arrays with (36.4 ± 4.7) nm and the highest background with (50 ± 7) nm at 5*g* is 13.6 nm. In up-side-down orientation, the difference between the lowest background of 1*g* arrays with (37.6 ± 3.9) nm and the highest background with (53.6 ± 4.3) nm at 10*g* is 16 nm.

Different 1g comparisons with g-levels

p-value	Array1	5.4*g*	10*g*
5.4*g*	< 0.001		
10*g*	0.09	< 0.001	
19.5*g*	0.013	< 0.001	< 0.001

Table S1: Cells on Array1 were exposed to 1*g* and showed the lowest force distribution, see Fig. 2. Shown are p-values of traction force per pillar comparisons of Array1 with different g-levels.

p-value	Array4	5.4*g*	10*g*
5.4*g*	< 0.001		
10*g*	< 0.001	< 0.001	
19.5*g*	0.07	< 0.001	< 0.001

Table S2: Cells on Array4 were exposed to 1*g* and showed the highest force distribution, see Fig. 2. Shown are p-values of traction force per pillar comparisons of Array4 with different g-levels.

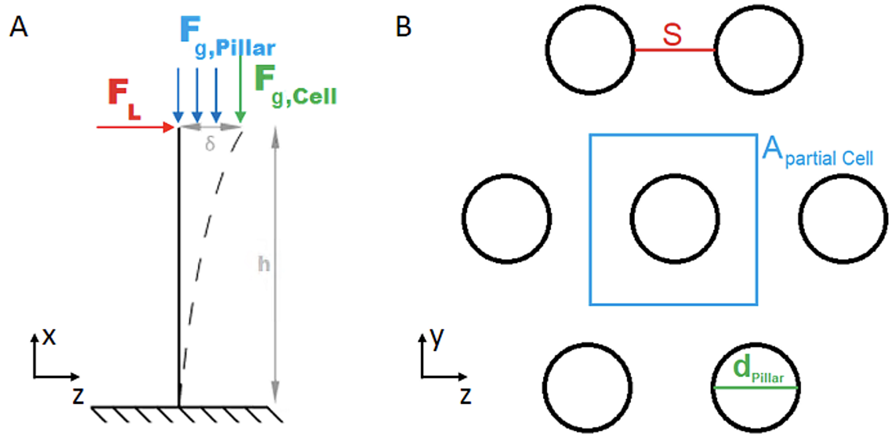

Figure S1: A: Micropillar is described by a cantilevered beam with the length, h. A cell sitting on the upper pillar end produces a lateral force, F, and causes a deflection, δ, of the pillar. In addition, gravitational forces increase the weight of the cell, $F_{\mathrm{g,Cell}}$, and the pillar, $F_{\mathrm{g,Pillar}}$, causing a higher deflection. B: Schematic top view of the hexagonal micropillar array. S is the spacing, d_{Pillar} the diameter of the pillar and $A_{\mathrm{partial-Cell}}$ the partial area of the cell that acts on one single pillar.

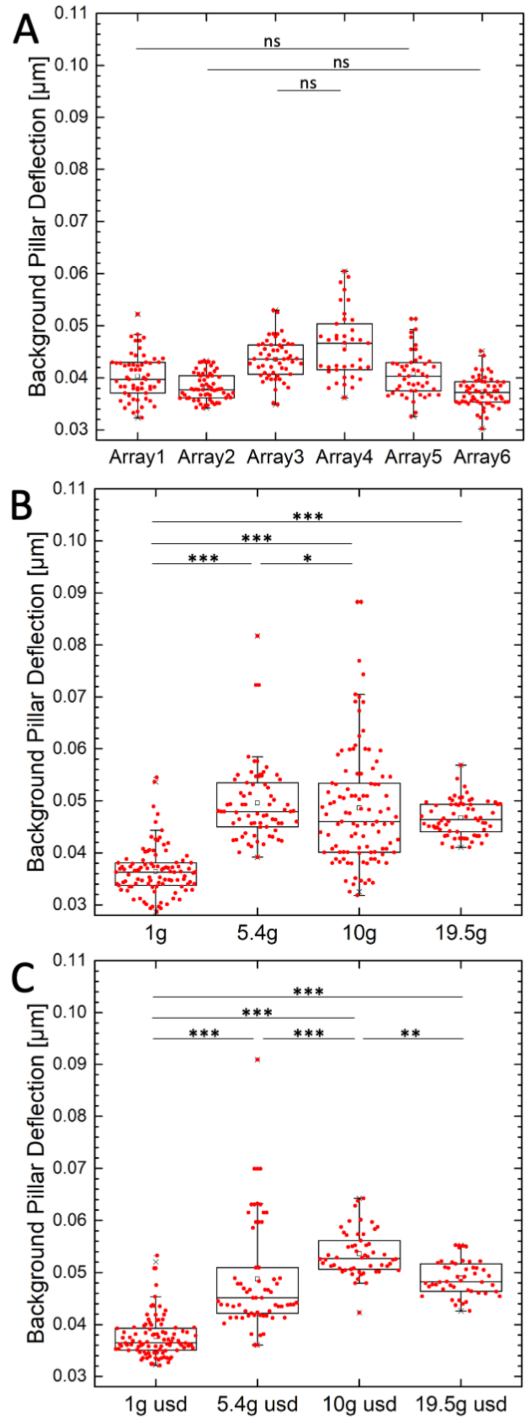

Figure S2: Background pillar deflection. A: 1*g* arrays in upright orientation corresponding to Fig. 2; mean deflections: Array1: 40.3±4.3 nm; Array2: 38.3±2.6 nm; Array3: 43.5±3.8 nm; Array4: 46.2±6.4 nm; Array5: 40.8±4.4 nm; Array6: 37.3±2.8 nm. B: upright orientation; 1*g*: 36.4±4.7 nm; 5.4*g*: 50±7 nm; 10*g*: 49±11 nm; 19.5*g*: 46.7±3.4 nm. C: up-side-down orientation; 1*g*: 37.6±3.9 nm; 5.4*g*: 49±10 nm; 10*g*: 53.6±4.3 nm; 19.5*g*: 49.0±3.3 nm. 10 nm is equal to 412 pN. Dunn's test of multiple comparisons following a significant Kruskal-Wallis test was performed. Each data point represents one analyzed cell.

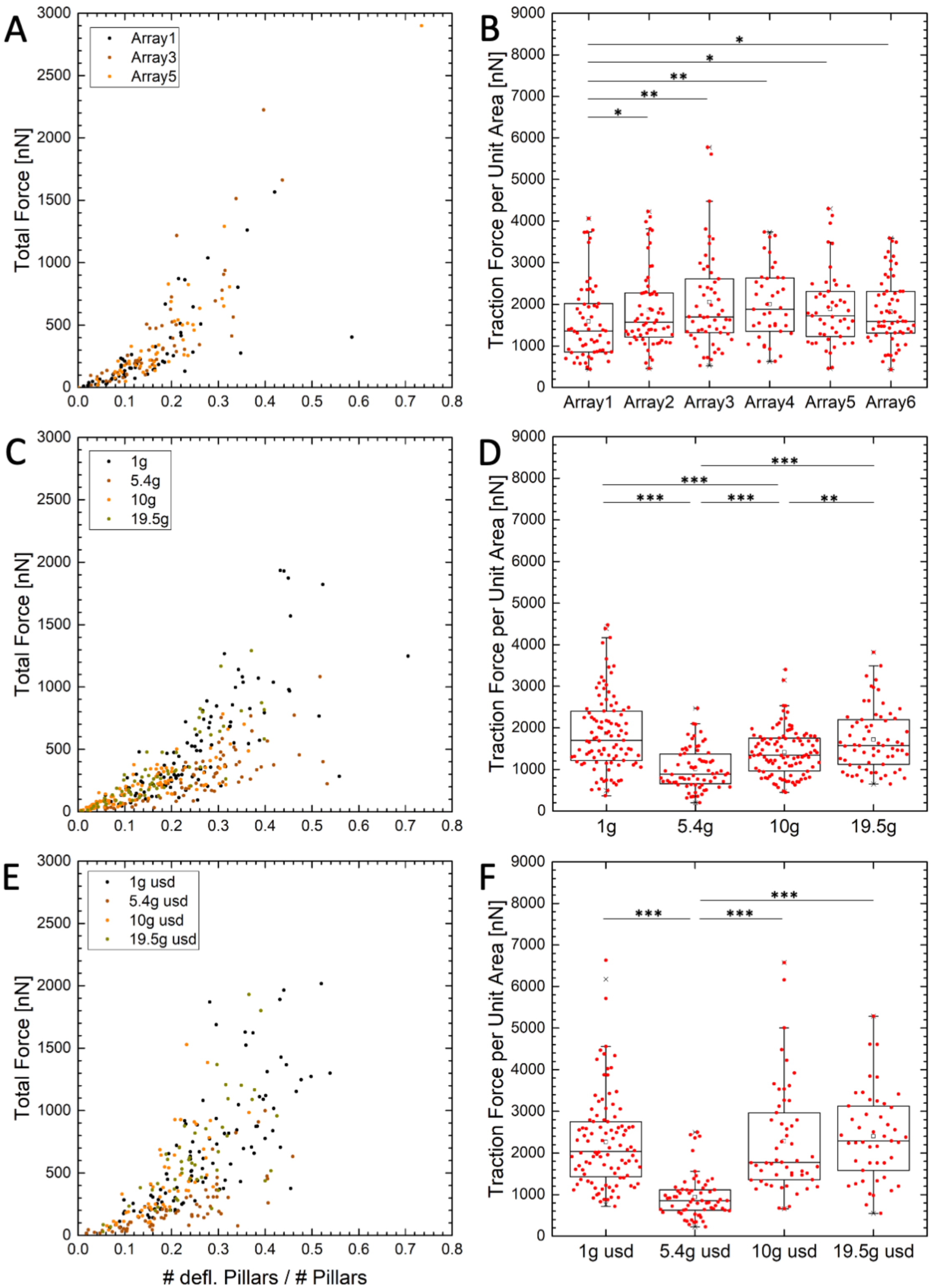

Figure S3: A,C,D: Total absolute force over ratio of number of deflected pillars to number of pillars underneath the cell. B-D-F: Force per unit area. B: 1*g* arrays corresponding to Fig. 2; mean values: Array1: 1.6±0.9 mN; Array2: 1.9±0.9 mN; Array3: 2.1±1.2 mN; Array4: 2.0±0.9 mN; Array5: 1.9±0.9 mN; Array6: 1.8±0.8 mN. D: upright orientation; 1*g*: 1.9±0.9 mN; 5.4*g*: 1.01±0.49 mN; 10*g*: 1.4±0.6 mN; 19.5*g*: 1.7±0.7 mN. F: up-side-down orientation; 1*g*: 2.3±1.1 mN; 5.4*g*: 0.9±0.4 mN; 10*g*: 2.3±1.3 mN; 19.5*g*: 2.4±1.1 mN. Dunn's test of multiple comparisons following a significant Kruskal-Wallis test was performed. Each data point represents one analyzed cell.