Electronic Supplementary Material (ESI) for Soft Matter.

Supplement to "Contractile Cell Forces Deform Macroscopic Cantilevers and Quantify Biomaterial Performance"

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This supplement serves to understand the employed surface stress measurement procedure in more detail and determine potential sources of measurement errors.

S1 Details on Setup Calibration before each measurement

S1.1 Linearity Calibration

The contractile forces by cells on top of a cantilever were determined by measuring the deflection of a laser beam pointed towards the bottom side of the cantilever. The reflected beam was detected by a position sensitive detector (PSD). The linearity of the detector was confirmed by pointing a laser beam directly onto it at conventionally measured (actual) positions and comparing the readout with the known real position of the laser. The readout contained a sum and a difference signal of a one dimensional photo detector over a total length of 1 cm. The nominal (detected) position was calculated as x = DIFF/(2*SUM) + 0.5. Figure S1 displays the resulting curve and a linear fit. It becomes evident that a linear fit is very accurate but that the scaling and offset has to be adjusted. This is obvious, since background light will increase the sum signal while reducing the absolute of the difference signal. Therefore, each measurement started by measuring the readout of the PSD at the positions 0.2 cm and 0.8 cm and rescaling the afterwards measured readout to these values.

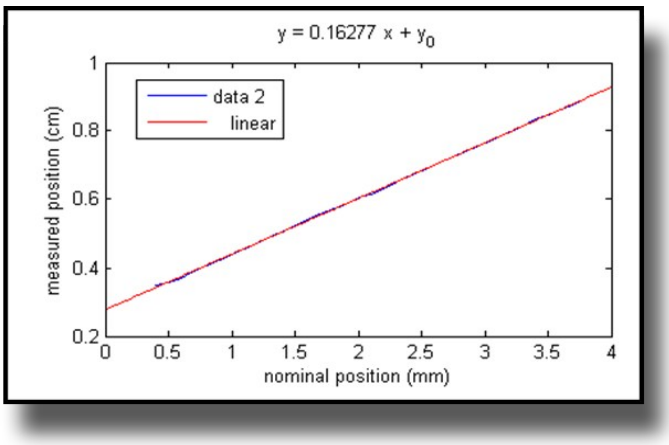

Fig. **S1**: Linearity calibration of the utilized position sensitive detector.

S1.2 Noise and Drift

We also tested the behavior of the setup on a cantilever without cells. The results are displayed in figure S2. The left graph shows the signal from an undisturbed cantilever over the course of one hour. From the moment of clamping the cantilever in, it requires about 10 minutes relaxation time to adjust to the medium as new environment. We attribute this to slight temperature differences and relaxation of the clamping. In eventual measurements, this fact was addressed by waiting 15 minutes before starting a measurement and by beginning each measurement with 100 seconds disturbance free data to confirm that relaxation time was sufficient. The second factor we determined was the amount of drift. This was captured over a whole hour, while eventual measurements only have a duration of less than 10 minutes. The determined drift accounts for less than 1 % of the effect measured by cell detachment. We also assessed the noise of the system and conclude that the signal to noise ratio is 0.3 %.

S1.3 Stability

A critical moment in the measurement is the addition of trypsin to the system. Therefore we tested various ways of disturbing the cantilever and analyzed if a permanent effect is detectible. The right side of figure S2 displays the position of the laser spot after adding fluid with a syringe or a pipettor at different speeds and even with and without touching the cantilever, which of course was strictly avoided during an actual measurement. We found that even in the touching case, disturbance accounts for less than 2 % of the measured difference in deflection by cell detachment. For the measurements we chose the most gentle technique of adding trypsin with a syringe and at safe distances between the cantilever and syringe tip.

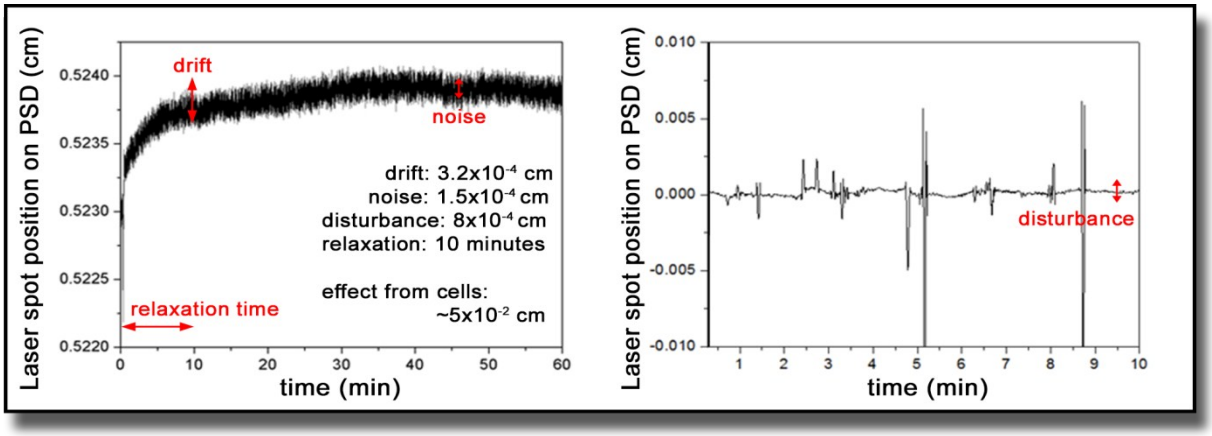

Fig. **S2** Noise and drift of the undisturbed cell-free cantilever (left) and reaction to various disturbances (right).

S1.4 Cell Detachment Confirmation

After above mentioned linearity calibration of the PSD readout, but before clamping the cantilever into the setup, its surface was investigated under a fluorescence microscope located right next to the setup. After the whole measurement procedure, it was imaged again to confirm that all cells were detached. Figure S3 shows typical micrographs before and after the trypsin treatment, showing that the cells grow in monolayers and are fully removed from the cantilever after measurement.

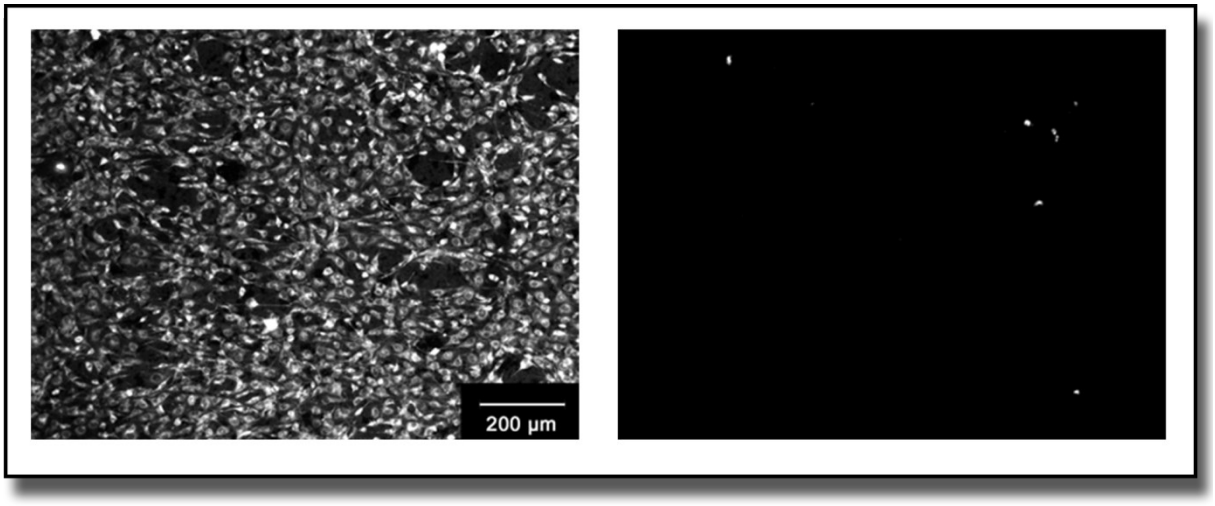

Fig. **S3** Fluorescence micrographs of the cantilever before and after measurement, showing a monolayer of fibroblasts before and the presence of less than 10 cells per mm² after trypsin treatment.

S2 Confirming Cells as Origin of Cantilever Deflection

We put high priority on confirming that our results are indeed the effect of the detaching cells and not an artifact of the technique. To address this issue, we kept the cells in DMEM during the measurement and never removed the cantilever from the setup during a comparison of the laser beam deflections. Therefore effects from the pre-bending of the cantilever and from the bending due to cells weight are negligible, since cantilever pre-bending is present to the same amount before and after trypsin treatment and the weight density of cells does not differ significantly from that of the surrounding medium.

We furthermore performed measurements in which cells were grown on the bottom side of the cantilever. These measurements are prone to large errors and should not be used to determine eventual results. The reason for this is a much higher disturbance of the laser beam during the measurement since the trypsin-water turbulences now directly occur in the light path. Also the glued reflector plate was left void of cells to avoid phototoxic effects and to maintain its reflectivity, which could also influence the results. Despite these restrictions, we measured that cantilevers with cells on the opposite side bent by a similar absolute value, but in the opposite direction. For titanium with cells on top, an average surface stress of $\sigma = (610 \pm 130)$ pN/µm^2 was measured. The measurements with cells on the bottom side of titanium gave $\sigma = 1508$ pN/µm^2 and $\sigma = 493$ pN/µm^2 in two independent exemplary measurements.