Supporting information

Spatial patterning of nanofibrous collagen scaffolds modulates fibroblast morphology

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S1: Topography of nanofibrous and smooth collagen scaffolds

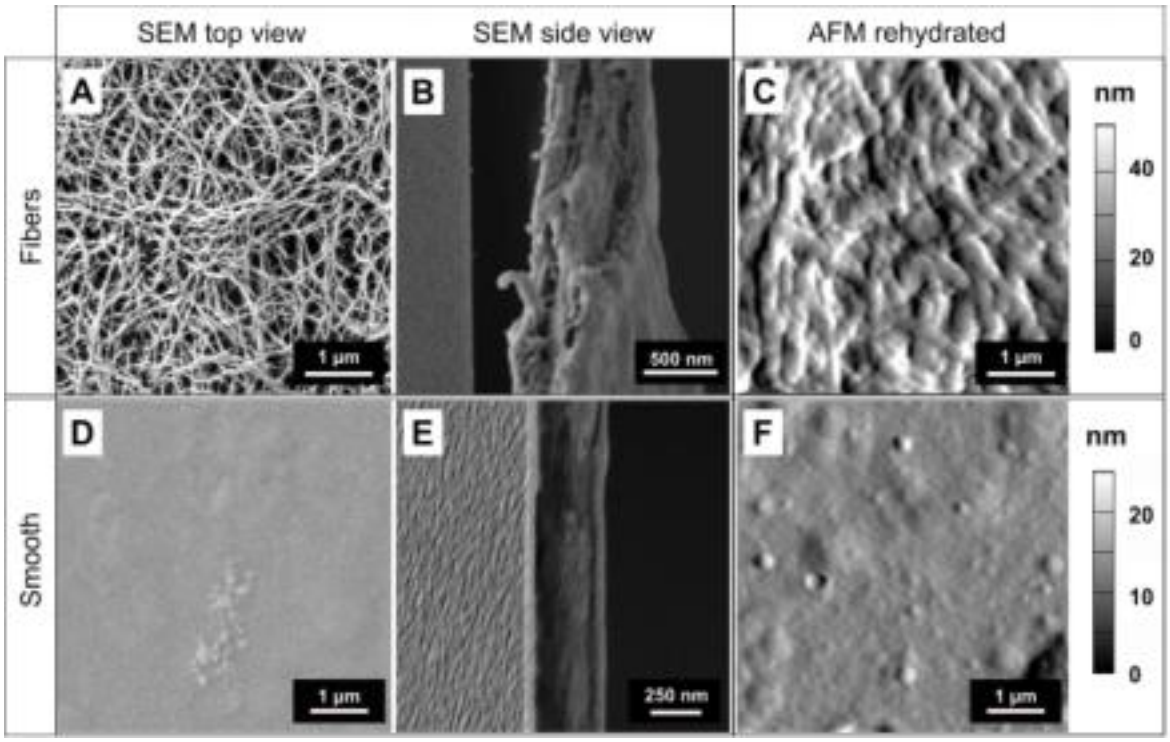

Figure S-1. *Topography of thin nanofibrous collagen (A to C) in comparison to smooth collagen scaffolds (D to F), both prepared with 0.5 mg ml^{-1} collagen. SEM images of dried scaffolds in top view show topographical differences between collagen nanofibers (A) and smooth collagen (D). SEM images of dried scaffolds in side view display the thickness of nanofibrous collagen (B) and smooth collagen (E). AFM scans show the deflection data of collagen nanofibers (C) and smooth collagen (F), which were both rehydrated in DMEM cell culture medium.*

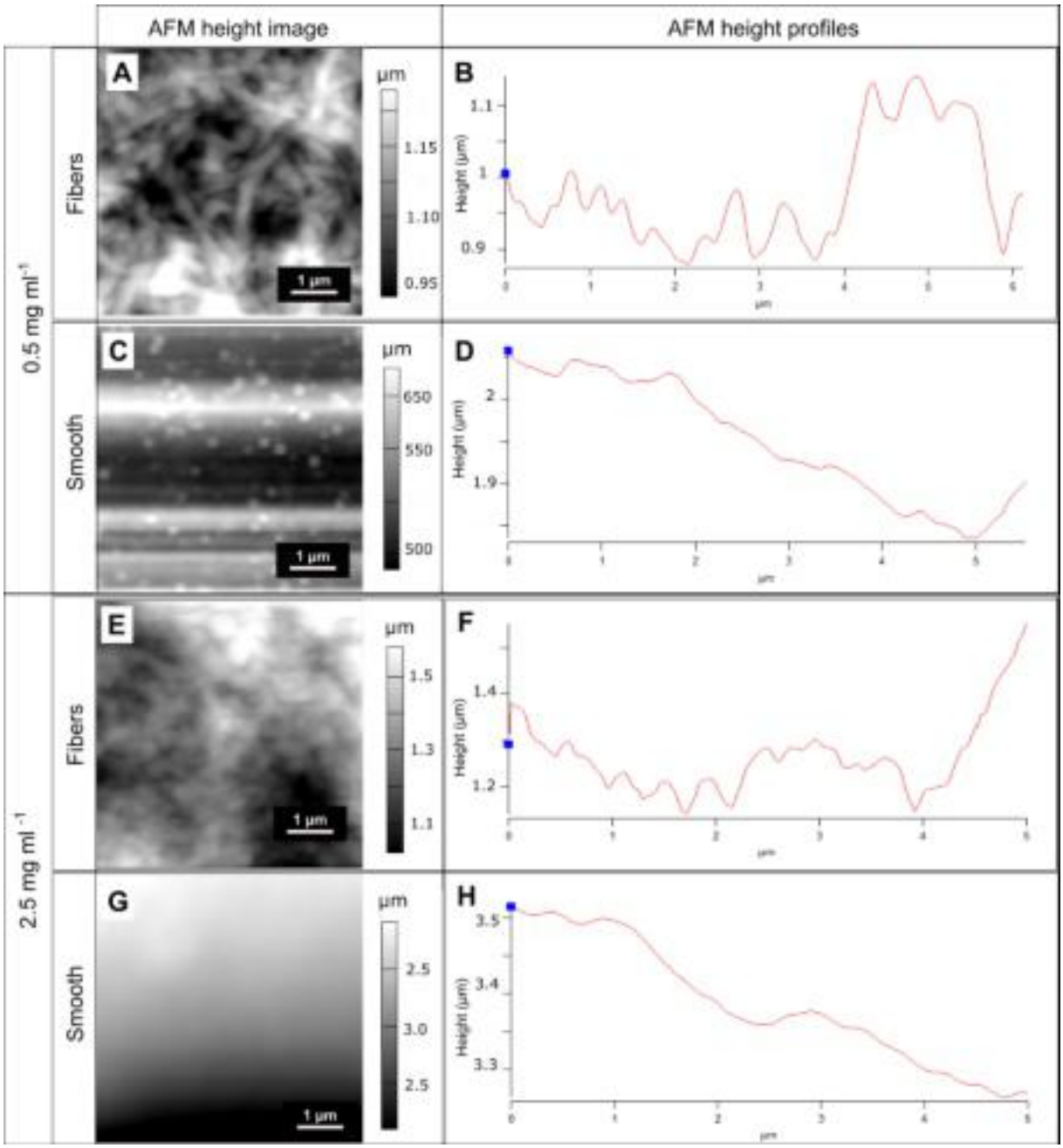

Figure S-2. *Roughness analysis of collagen scaffolds obtained from AFM height images and profiles for nanofibrous collagen prepared with 0.5 mg ml^{-1} (A and B) and with 2.5 mg ml^{-1} collagen (E and F) in comparison to smooth collagen prepared with 0.5 mg ml^{-1} (C and D) and with 2.5 mg ml^{-1} collagen (G and H). All height profiles were measured in contact mode with a scan rate of 1 line per second at 256 lines per frame (frame size 5 µm^2) and were subsequently averaged over a ROI of 1 µm^2.*

Table S-1. *Roughness value (Rq) of dried and rehydrated collagen scaffolds obtained from AFM analysis of nanofibrous and smooth collagen scaffolds. Scaffold roughness was found to increase with increasing collagen concentration as well as upon rehydration in DMEM cell culture medium.*

	0.5 mg ml^{-1}		**2.5 mg ml^{-1}**	
	Rq $_{dried}$ (nm)	**Rq $_{rehydrated}$ (nm)**	**Rq $_{dried}$ (nm)**	**Rq $_{rehydrated}$ (nm)**
Collagen fibers	39.9 ± 1.2	43.2 ± 3.8	46 ± 5.7	114.8 ± 87.5
Smooth collagen	8.8 ± 4.3	29 ± 5.4	27.3 ± 3.2	22.6 ± 6.6

S2: Stiffness of collagen scaffolds

An MFP-3D atomic force microscope (AFM, Asylum Research, Santa Barbara, CA, USA) was used to study the mechanical properties of fibrous and smooth collagen. First, the spring constant of the cantilever was calibrated by using a thermal tune method [1]. Subsequently, force curves were recorded in random positions of the substrates. Typically, a scan rate of 1 Hz was used, which corresponds to a maximum loading rate of 1 nN s^{-1} and a maximum force of 1 nN. We used an indentation depth of 100 nm. At least 3 independent samples were measured for each substrate type with an indentation depth of 100 nm. At least 100 force curves were recorded over a force map area of 5 μm × 5 μm in the form of grids.

Subsequently, the data analysis package IGOR (Wavemetrics, Lake Oswego, OR, USA) was used to obtain the Young's modulus. An average stiffness value was obtained from three independent experiments (n=3) performed on the respective collagen scaffolds. The Hertzian model for pyramidal tips was used to calculate the Young's modulus for each force curve within a force map.[2] The median of these values was considered as a representative Young's modulus per force map area.

S3: Viability of 3T3 fibroblasts on collagen scaffolds

To measure the cell viability we conducted a live/dead staining with calcein-AM (Thermo Fisher) and ethidium homodimer-1 (EthD-1, PromoCell GmbH, Heidelberg, Germany). 3T3 fibroblasts were cultivated on collagen scaffolds prepared with 0.5 mg ml^{-1} protein concentration and on glass slides. Dead cells were used as a positive control (PC), which were killed by incubation with 1 % 100 X Triton (v/v) (Carl Roth GmbH) in 1 x PBS for 30 min at 37 °C. All samples were washed with pre-heated 1 x PBS before immersing them with 2 µM calcein-AM and 4 µM EthD-1 dissolved in 1x PBS. All samples were incubated at 37 °C for 1 h. Subsequently, ten images with 20x magnification were recorded for each sample type using a Nikon Eclipse Ti fluorescence microscope. For dead cells an emission wavelength of 617 nm was used, and living cells were imaged at an emission wavelength of 395 nm.

The viability data obtained from the live/dead staining are presented as average ± standard deviation of all values obtained from four independent experiments (n = 4) performed with triplicates for each substrate type (see Figure S-3). Asterisks indicate the significance of differences between multiple groups of data (excluding the positive control) from statistical analysis carried out using ANOVA (* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$).

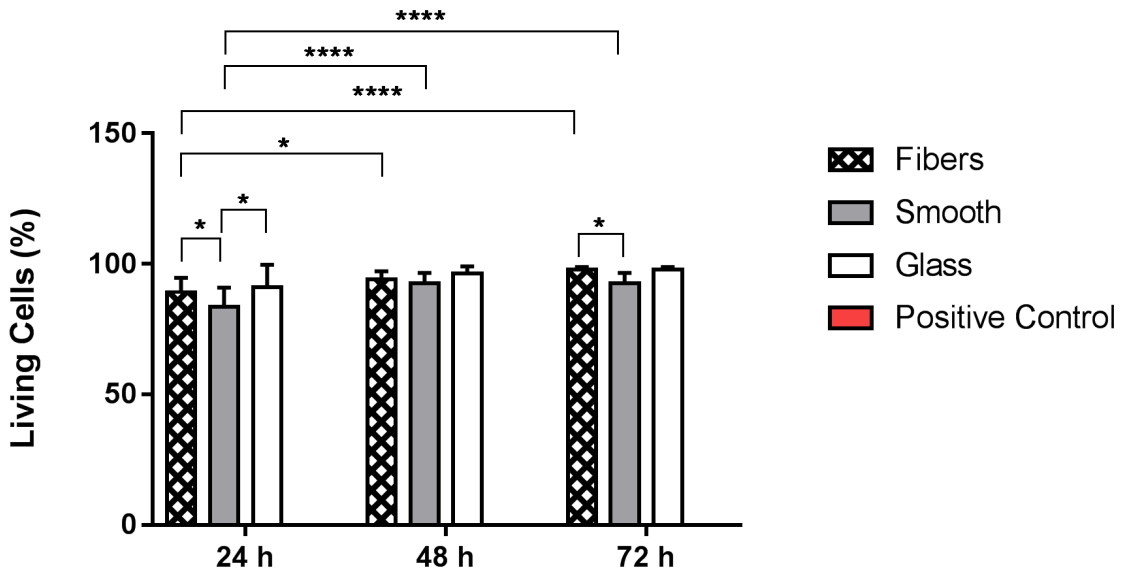

Figure S-3: *Percentage of viable 3T3 fibroblasts on different substrates obtained by live/dead staining). Percentage of viable fibroblasts on smooth and nanofibrous collagen,bothe prepared with 0.5 mg mL^{-1}, and on glass for 24 h, 48 h and 72 h. The positive control represents dead cells on glass with values too low to be displayed. n = 4 experiments were conducted with triplicates for each sample type. Data are presented with average ± standard deviation of replicates. PC is excluded from ANOVA-test. Significant differences indicated by * $p \leq 0.05$, **** $p \leq 0.0001$.*

S4: Stiffness of 3T3 fibroblasts on different collagen scaffolds

The fibroblast stiffness on different collagen scaffolds was measured with AFM using MLCT-BIO tips. To obtain average the cell stiffness over a large contact area, indentation depths between 200 and 500 nm were chosen so that the resulting values did not depend on local variations of the underlying cytoskeleton structure. After 24 h, cell samples were transferred to the AFM and constantly supplied with CO_2. The AFM tip was approached to each fibroblast in an area close to the cell nucleus, where the cell reaches its maximum height. A minimum of 17 cells per substrate type were analyzed with a minimum of 100 force curves in an area of 5 µm × 5 µm. Subsequently, the Young's modulus was calculated using IGOR and the Hertzian model (see section S2).

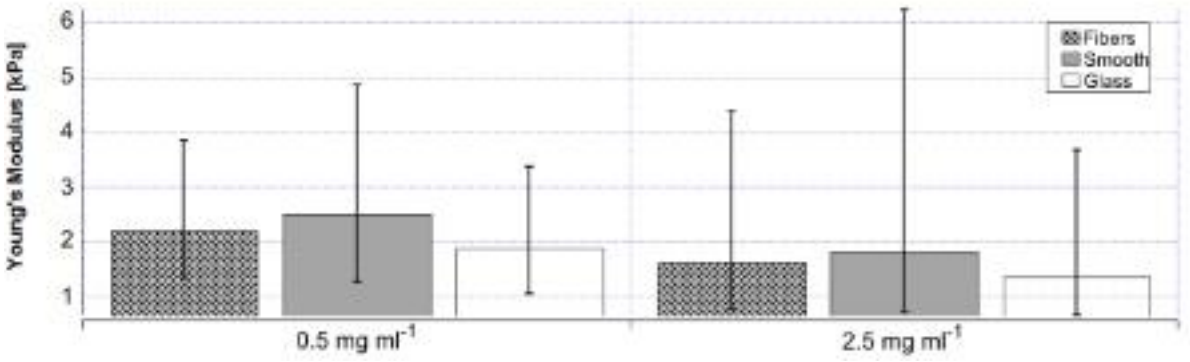

Figure S-4*: Young's modulus of 3T3 fibroblasts after 24 h cultivation on nanofibrous and smooth collagen in comparison to fibroblasts grown on glass. Collagen scaffolds prepared with 0.5 mg ml^{-1} are displayed on the left, and scaffolds prepared with 2.5 mg ml^{-1} are shown on the right. No variations in fibroblast mechanics were observed among the different substrate types.*

S5: Migration of 3T3 fibroblasts on different collagen scaffolds

To study the migration velocity of 3T3 fibroblasts in dependence of the underlying scaffold topography, histograms of the log(v) distribution were analyzed. Fig. S-5 exemplarily shows the histograms of nanofibrous and smooth scaffolds prepared with 2.5 mg ml^{-1} collagen in comparison to glass. Shoulders at low velocities are marked with a black circle. In these histograms, values of log(v) < 0.1 µm min^{-1} and log(v) > 10 µm min^{-1} were considered as artefacts, e.g. caused by non-migrating cells or by small air bubbles in the cell medium, and

were therefore excluded from the following Gaussian fit analysis. Subsequently, Gaussian fits were applied to the logarithm of the median migration velocities log(v). From the resulting fit peaks, we obtained the median fibroblast velocities for each scaffold type.

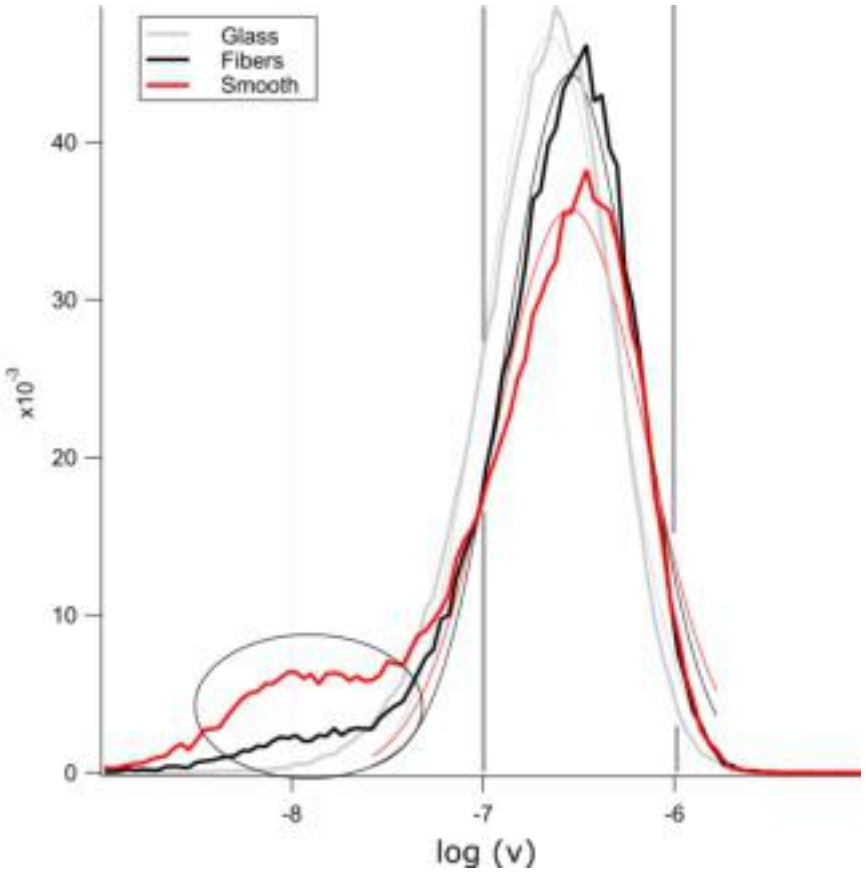

Figure S-5*: Histograms of 3T3 fibroblast velocities on different substrates show the pooled logarithm of the migration velocities for nanofibrous collagen (black) and smooth collagen (red) prepared with 2.5 mg ml^{-1} collagen in comparison to glass substrates (light grey). The black circle highlights the shoulders of the histograms at low migration velocities, which were excluded from Gaussian fitting by using the data between log(v) = 0.1 µm min^{-1} and log(v) = 10 µm min^{-1} (indicated by vertical grey lines). The maxima of the fitted peaks are shifted compared to the original histograms and were used for further statistical analysis.*

From Gaussian fitting we obtained the peak maxima for 3T3 fibroblasts on the different scaffold types (see Fig. S-6). By excluding values below 100 nm min^{-1} and above 10 µm min^{-1} the peak maxima in the pooled histograms (indicated by dashed lines) were shifted in comparison to the original mean data (solid lines). For subsequent statistical analysis, Cohen's d effect size of the pooled median fibroblast velocities was calculated based on the local Gaussian fit results. Consequently, only the velocities in the fits of the main peaks were

contributing to the calculation of the effect size, i.e. cell velocities from 100 nm min^{-1} to 10 µm min^{-1}.

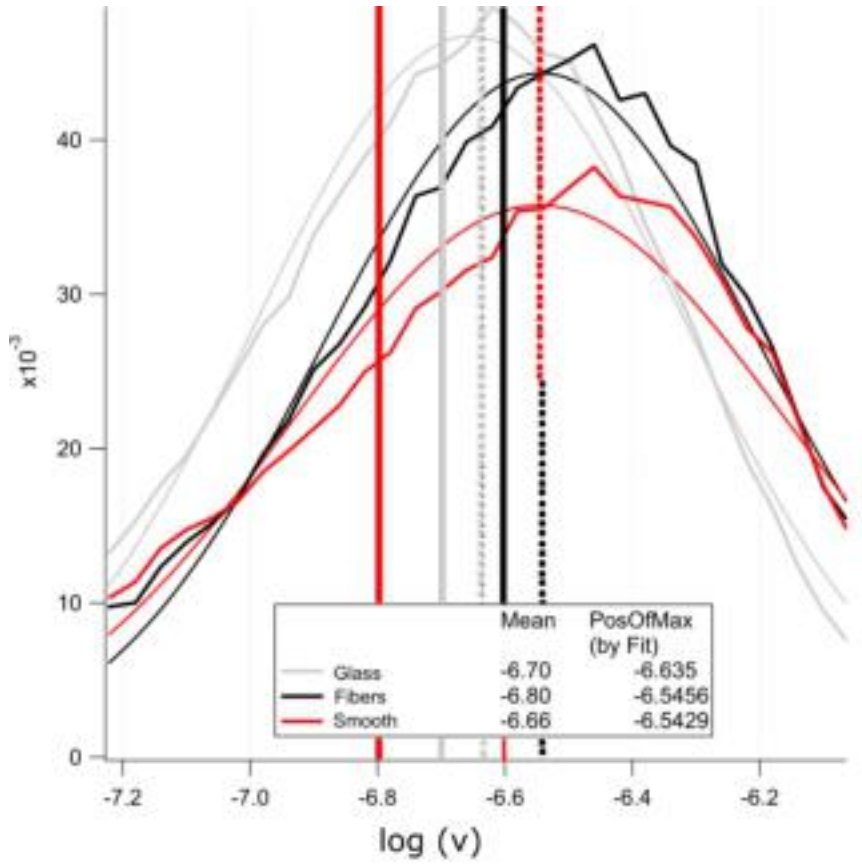

Figure S-6*: Zoom into the peak positions in the histograms of fibroblast velocities on different collagen scaffolds and on glass. Log(v) on nanofibrous collagen (black) and smooth collagen (red) prepared with 2.5 mg ml^{-1} collagen compared to glass references (light grey). Only data between log(v) = 0.1 µm min^{-1} and log(v) = 10 µm min^{-1} were analyzed by Gaussian fitting. The resulting peak maxima in the pooled histograms obtained by Gaussian fitting (indicated by the respective dashed lines) were clearly shifted in comparison to the original mean data (solid lines).*

References

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[2] F. Rico, P. Roca-Cusachs, N. Gavara, R. Farré, M. Rotger, D. Navajas, Probing mechanical properties of living cells by atomic force microscopy with blunted pyramidal cantilever tips, Phys. Rev. E - Stat. Nonlinear, Soft Matter Phys. (2005). https://doi.org/10.1103/PhysRevE.72.021914.