Supporting Information

Solvent-Induced Nanotopographies of Single Microfibers Regulate Cell Mechanotransduction

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EXPERIMENTAL SECTION

Materials

All chemicals are used as received without any further purification. Polycaprolactone (PCL) with an average molecular weight of 80 kDa (Sigma-Aldrich, Germany) is dissolved in different single and binary solvent systems. The solvent properties applied in this study are shown in **Table 1**. Chloroform (CF), dichloromethane (DCM), and toluene (TOL) are chosen as good solvents, acetone (AC), acetic acid (AA), and dimethylformamide (DMF) as partial solvents, and dimethyl sulfoxide (DMSO) as insoluble solvent. Binary solvent systems are prepared at 25-75, 50-50, and 75-25 (vol-vol%) mixing ratios.

Solution preparation and the spinning process

The polymer solutions for SAS are prepared by dissolving PCL in single or binary-solvent systems to obtain 15 wt% or 17 wt% polymer concentration, depending on the required fiber diameter. For all solutions, solvent is added to a pre-weighed amount of PCL and magnetically stirred at room temperature (RT ~ 22-25 °C) until complete dissolution of the polymer (at least 1h). Syringes (1 mL) are filled with the prepared solution and the feed rate (Q) is controlled by a programmable syringe pump (Aladdin, WPI) between 50 and 150 µL/hr. As the solution is pushed through the needle (21G), it forms a hemispherical shape at the tip, which is manually drawn towards the rotating cylindrical drum (radius = 2.5 cm) that collects aligned microfibers. The collector is placed on top of a translational linear stage with adjustable speed while the collector's rotational speed (V_R) is controlled via a digital speed controller and set at 50, 100, or 150 rpm. The needle's tip to collector distance (TIP) is set at 5 cm in the vertical direction. All experiments are performed at room conditions with a temperature of 22-25 °C and relative humidity of 42-45 %. Fibers are collected on 15 mm cover glasses, coated with poly (ethylene glycol) (PEG) to restrict unspecific interaction of cells with the glass substrate.

The jet linear velocity in SAS

In the case of SES, depending on the applied voltage and the solvent properties, the average linear velocity of the jet can reach up to 15 m/s.[1] However, as there is no columbic force in the case of SAS, the polymeric jet speed is equal to the linear velocity of the rotating drum.

The rotational and the linear velocity are related by Equation 1:

Eq. 1: $v = 2\pi r\omega$

Where v is the linear velocity of a drum with radius r and a rotational velocity of ω. Therefore, the maximum linear velocity of the jet used in this study can be calculated as:

$r = 0.25\ m$

$\omega = 150\ rpm$

$v = 2\pi \times 0.01 \times 150 \times \frac{1}{60} = 0.16\ m/s$

Fiber characterization

To measure the viscosity of the single or binary solvent systems (50-50), a rotational rheometer (TA Instruments HR-3 Rheometer PHR3, Waters, Milford USA) with a cone-plate geometry of 20 mm diameter and cone angle of 2˚ is applied. The temperature is maintained at 25˚C for all measurements. A logarithmic frequency sweep program is run from 0.1 to 100 s^{-1} with an increment of 3 s^{-1}. Viscosity values are taken at a shear rate of 1 s^{-1}. A solvent trap is used to prohibit evaporation of the solvents during the measurement.

The surface topography and the diameter of the fibers are analyzed using field emission scanning electron microscopy (FESEM- Hitachi S4800, Japan). Prior to imaging, fibers are firmly adhered to aluminum stubs and sputter coated with gold-palladium. ImageJ (1.51 W, NIH, USA) software is used to measure the pore width, length, and the groove width. All the FESEM figures are imported in ImageJ as “binary”, black and white, images. A threshold range is set to remove the background. The measurement area (fiber’s border) is set and the particle analyzer is used, while ellipsoids are fitted to the pores. The minimum and maximum pixel area size is set to 0 and infinity, respectively, to include all pixels that are in the region of interest. Major and Minor values are the primary and secondary axis of the best fitting ellipse and are defined as the pore length and width, respectively. In the case of groove fibers, the Minor value is set as the groove width.

The static water contact angle of the different samples is determined with the sessile drop method using the Kruss Drop shape analyzer (DSA 2). The measurements are performed both

perpendicular and parallel to the orientation of the fibers. Seven droplets of distilled water are placed randomly on the fibers with different surface topographies (n=3). Once the drop is placed on the fiber surface, a settling time of 20 sec is allocated before the images are taken.

Crystallization analyses are carried out using differential scanning calorimetry (DSC), wide-angle X-ray scattering (WAXS), and incidence wide-angle Scattering (GIWAXS). DSC thermograms are obtained using the PerkinElmer (DSC 8500) differential scanning calorimeter under N_2 gas flow (50 mL/min) at a scanning rate of 10 °C/min from 0 to 150 °C (first heating), from 150 to 0 °C (first cooling), and again from 0 to150 °C (second heating). The melt enthalpies of the different samples are obtained from the first heating scans. The degree of crystallinity (X_c) is calculated based on Equation 2 where ΔH_m is the enthalpy of melting and ΔH_m^0 the melting enthalpy of 100% crystalline PCL (139.5 J/g).[2]

$$\text{Eq. 2:}\quad Xc = \frac{\Delta H_m}{\Delta H_m^0} \times 100$$

WAXS and GIWAXS are done using an Empyrean setup from PANalytical. A Cu x-ray tube (line source of 12×0.04 mm^2) provides Cu Ka radiation with l=0.1542 nm. A geometry with a parallel incident beam is used, which is especially useful at low angles (below 10°). A divergence slit of 1/8° is set to illuminate part of the parabolic graded multilayer system (Göbel mirror). The latter converts 0.8° from the divergent beam into an almost parallel beam (divergence ≤ 55 mdeg). Source and detector moved in the vertical direction around a horizontally fixed sample at the center of a phi-chi-z stage. The scattered signal is recorded by a pixel detector (256×256 pixels of 55 μm) as a function of the scattering angle 2Θ. The diffraction profiles are fitted using the Highscore software and the diffraction peaks are identified and used to measure the crystallinity degree.

To determine the elastic moduli of SAS fibers with different topographies, atomic force microscopy (AFM) (Bruker, Dimension Icon FastScan System) with nanoindentation is used. A s-Qube® colloidal probe (Nano and more, CP-PNPS-SiO-A-5) with a 2 μm (+/- 5%) diameter silicon dioxide sphere on a triangular gold coated cantilever is calibrated in air using the thermal resonance method, built into the AFM software (K = 0.2256 N/m). An indentation is applied at 1 μm/s with a maximum force of 10 nN over a line with a length of 500 nm in 100 nm steps with

five measuring points. The elastic moduli are measured by fitting the Hertz model using a non-linear fit.

Cell culture and immunostaining

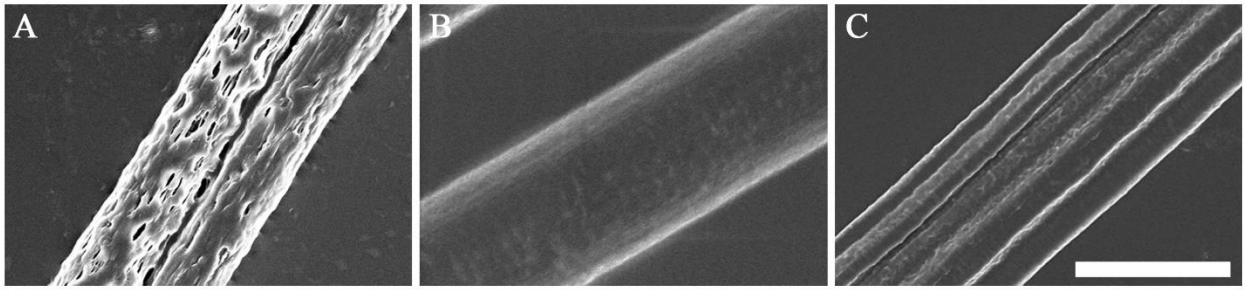
Samples are UV sterilized for one hour, submerged in 70% ethanol for 10 minutes, and washed twice for 10 min with sterile PBS prior to cell seeding. A suspension with mouse-derived L929 fibroblasts (40,000 cells/mL) is prepared in RPMI media, supplemented with 10 vol% fetal bovine serum (FBS, Biowest) and 1 vol% antibiotic-antimycotic (Gibco™). A cell suspension of 1 mL is placed on top of the fibers and incubated at 37˚C and 5 % CO_2 for 72 h. To evaluate the effect of fiber topography on the proliferation activity of cells, a proliferation assay using (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) (MTS, Promega) is performed. The cells (40,000 cells/well) are cultivated for 24 and 72 h, after which the samples are transferred to a new well with MTS solution and incubated for 1 h at 37 °C. Subsequently, the absorption of 100 µL of the MTS solution is measured in a 96 well plate at a wavelength (λ) of 490 nm using a microplate reader (Biotek® Synergy HT, USA). The obtained values are normalized to those measured for smooth fibers.

After cultivation, all samples are washed for 15 min with PBS and fixed with 4 wt% paraformaldehyde (AppliChem) for 30 min, followed by three washing steps (10 min) with PBS. Cells are permeabilized with 0.1 % Triton X (Sigma Aldrich) for 5 min and 4 wt% BSA is used as a blocking agent for 1 h followed by the addition of the primary antibodies against vinculin (Sigma-Aldrich, 1:100), Phalloidin-iFluor 488 (abcam 1:1000), YAP-647 (Santa Cruz Biotech, 1:100), or MRTFA-488 (Santa Cruz Biotech, 1:100), which are incubated for 3 h at RT. After washing three times for 15 min with PBS, secondary antibodies (Alexa Fluor 488 anti-rabbit or Alexa Fluor 633 anti-mouse (Thermo Fisher Scientific, 1:100)) are incubated for 1 h at RT, after which DAPI is added for 30 min. Finally, samples are washed three times for 15 min with PBS and stored at 4°C. Confocal laser scanning microscopy is performed with a Leica SP8 Tandem Confocal system, equipped with a white light laser (WLL). Depending on the stained dye, samples are excited with the appropriate wavelengths using the WLL or a photodiode 405 in the case of DAPI. Emission is detected with the PMT (photomultiplier tubes) detector for DAPI, or the HyD (Hybrid detector) for anti-vinculin, YAP, and MRTFA.

In order to define the nucleus and the cytoskeleton aspect ratio, the fluorescent images of cell nucleus, stained with DAPI, and the cell cytoskeleton, stained with Alexa flour 488, are imported to ImageJ software as "binary" images and the same procedure as described above for the fiber characterization is used to measure the Major and Minor values. The aspect ratios are defined as the Major/Minor. A minimum of 20 cells per condition is analyzed. The intensity analysis for MRTFA and YAP is also performed using the ImageJ software. A circular ROI with an area of ~ 4 μm^2 is placed randomly in the nucleus and the cytoplasm of the cell, respectively, and the mean fluorescence intensity (total intensity/number of pixels) is determined. Data are plotted as the ratio of the mean fluorescence intensity in the nucleus to the mean fluorescence intensity in the cytoplasm ($F_{Nucleus}/F_{Cytoplasm}$). A minimum of 40 cells per condition is analyzed.

Statistical analysis:

All data are represented as mean ± standard deviations of at least three replicates. Statistical significance is determined by the one-way analysis of variance (ANOVA) using OriginPro 2016G software for $p < 0.05$.



D

Fiber Morphology	T_m(°C)	ΔH_m(J/g)	FWHM (°C)	X_c(%)	
Before SAS	60.38 ± 0.31	65.96 ± 0.21	9.28	47.28 ± 0.15	*
Smooth	59.50 ± 0.28	69.89 ± 0.25	5.46	50.10 ± 0.18	
Grooved	59.58 ± 0.03	70.41 ± 0.31	5.22	50.48 ± 0.22	
Porous	59.33 ± 0.25	71.53 ± 0.14	4.76	51.28 ± 0.10	

E

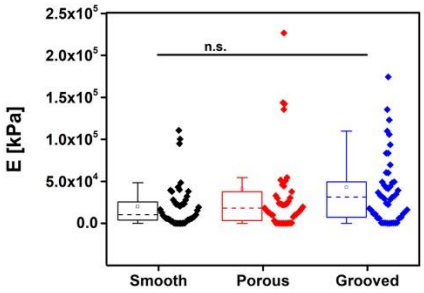


Figure S1: A) Porous fibers (PCL 15 wt%, 50-50 CF-DMSO) fabricated using a small needle (27G). B-C) Smooth (50-50 CF-AA) and grooved (75-25 CF-DMF) fibers with high PCL concentration (17 wt%). D) The thermal properties of PCL before and after SAS: T_m (°C) melting temperature, ΔH_m enthalpy of melting, the full width at half maxima (FWHM), and Xc (%) degree of crystallinity calculated from the first heating curve. E) The elastic modulus of SAS fibers measured using AFM demonstrating no significant difference between different fiber topographies. Scale bar: 10 µm.

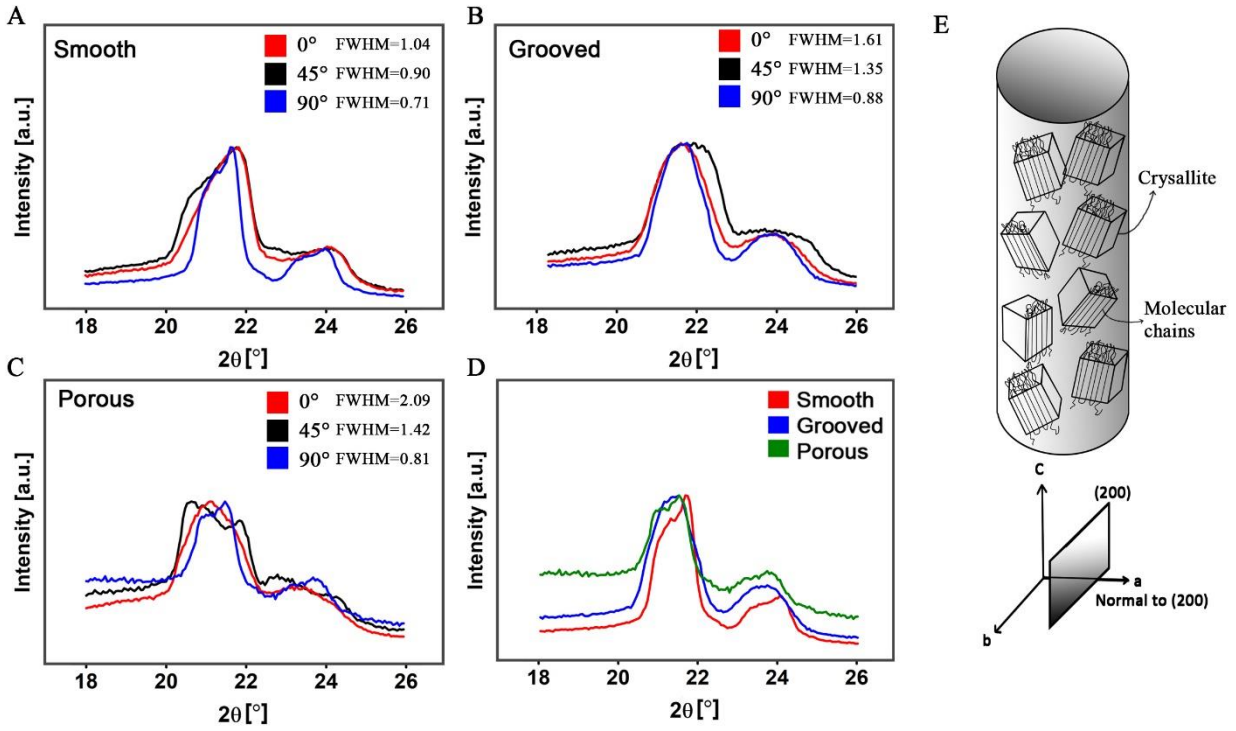


Figure S2: A) The GIWAXS measurements performed at three different angles (0°, 45°, 90°) in respect to the fiber orientation and beam direction for smooth (A), grooved (B) and porous (C) fibers. D) The GIWAXS graph of SAS fibers at 90° angle. E) Schematic of crystal lattices in a SAS fiber indicating that the c-axis of the crystal lattices formed by the molecular backbone is preferably oriented along the fiber axis.

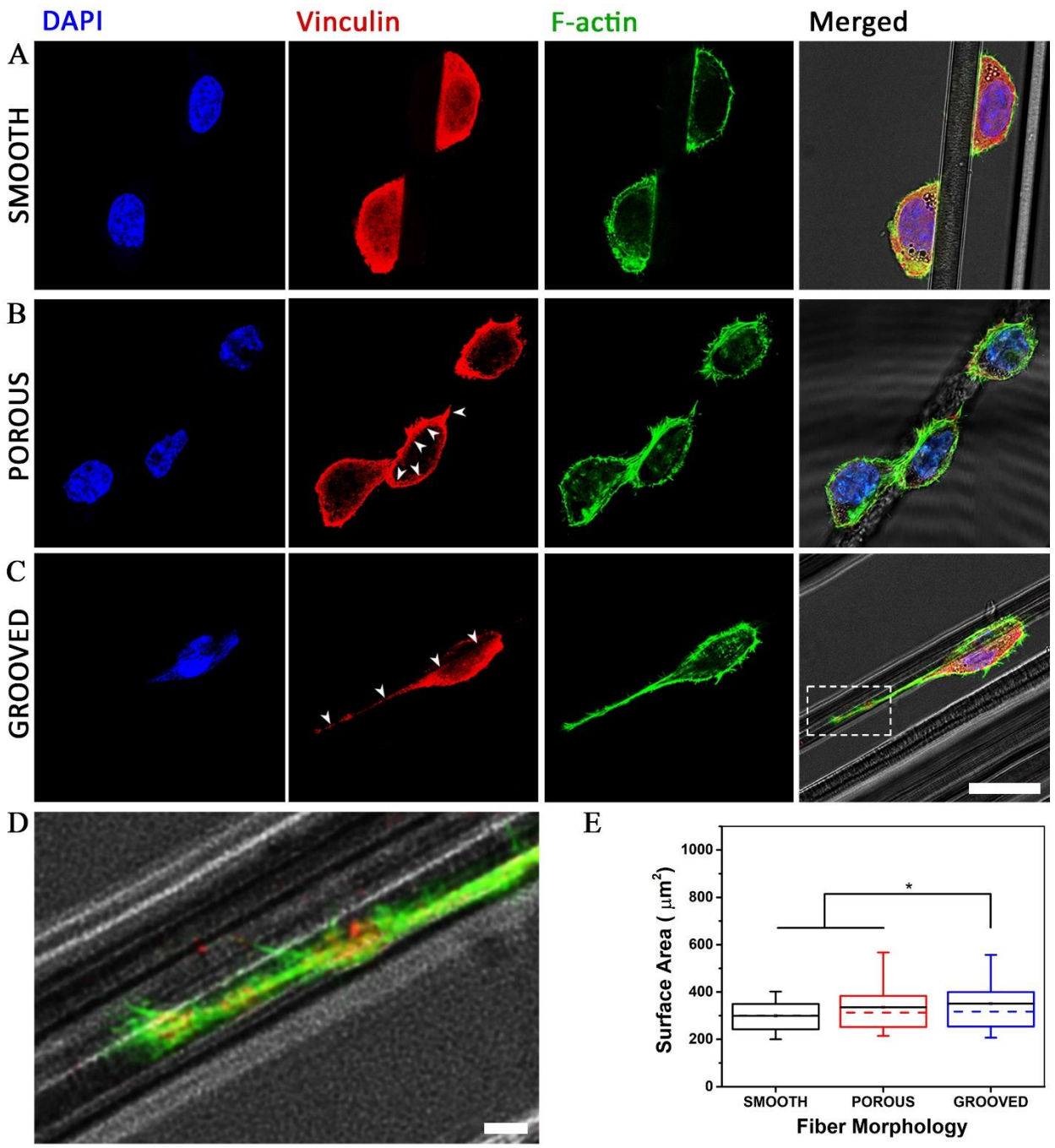


Figure S3: Fiber surface topography influences fibroblast actin filament elongation and the vinculin distribution: A) smooth, B) porous, and C) grooved. Small arrows indicate the vinculin expression areas. Scale bar: 20 μm. The white dash box in C is magnified in D. E) The surface area of cells depending on fiber topographies. Scale bar: 2 μm.

References:

1. Buer, A.; Ugbolue, S. C.; Warner, S. B., Electrospinning and Properties of Some Nanofibers. *Text. Res. J.* **2001,** *71* (4), 323-328.
2. Wang, X.; Zhao, H.; Turng, L.-S.; Li, Q., Crystalline Morphology of Electrospun Poly(E-Caprolactone) (Pcl) Nanofibers. *Ind. Eng. Chem. Res.* **2013,** *52* (13), 4939-4949.