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Supplementary data for

Surface directed modulation of supramolecular gel properties

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1. Methods

1.1. Materials

Clear glass vials 6 mm ID, 11.6 x 32 mm, 1.5 ml were purchased from Sigma-Aldrich. 13 mm diameter borosilicate microscope cover glasses (Lot: 30473832) were purchased from Marienfeld GmbH and Co KG, Germany.
Sulphuric acid 95-97% w/v BP analytical grade was purchased from Sigma Aldrich (Lot: SZBE2510V). Hydrogen peroxide 100 volumes >30% w/v laboratory reagent grade were purchased from Fisher Chemicals (Lot 1525019). Methanol HPLC grade (Lot 1541331), Acetone HPLC grade (Lot 1413194), Propan-2-ol analytical reagent grade (Lot 1416700), Toluene analytical reagent grade (Lot: 1529541) were purchased from Fischer Chemicals UK. Triethoxyphenylsilane (purity 98%) was purchased from Aldrich Chemistry UK (Lot: #MKBR4637V). All water used was milli Q (18.2 MΩ·cm) ultrapure water.

1.2. Experimental procedures

1.2.1. Surface modification

1.2.1.1. Preparation of hydroxyl- surfaces

Microscope cover glasses were placed onto a metal rack and were sonicated sequentially in methanol, acetone and propan-2-ol for two minutes in each solvent using Ultrawave Sonicator, Scientific Laboratory Supplies (Lot 37474994). After sonicating, the cover glasses were blow dried using pressurised air. The slides were then treated with piranha solution (3:1 of 97% H_2SO_4 : 30% H_2O_2) for 30 minutes. The cover glasses were rinsed using water and blow dried using pressurized air. The piranha etched vials were prepared following the same procedure. The same procedure was followed for the vials.

1.2.1.2. Preparation of phenyl- surfaces

Freshly piranha etched slides were placed in a metal rack and immersed in a 1% v/v triethoxyphenylsilane solution and left to react in an oven at 70^{o}C for one hour. The cover glasses were then rinsed sequentially with toluene, acetone and water and blow dried using pressurised air.
To silanise vials after piranha cleaning, the glass vials were fully immersed in a 10% v/v triethoxyphenylsilane solution in toluene under the same conditions and followed by the same washing procedure outlined above.

1.2.2. Gel preparation

1.2.2.1. C_{14}-cytidine.

To form the gelator stock solution 2.5 mg C14-cytidine and 150 µl ethanol were added into a glass vial, the solution was sonicated for 2 minutes and then heated at 60^{o}C until the gelator was fully dissolved.

1.2.2.2. Gel film formation

The modified glass slides were heated on a heating mantle to 60°C. 9 µl of the hot gelator stock solution (60°C) was pipetted onto the modified glass slides using a micropipette. This was immediately followed by the addition of 21 µl of water (also heated to 60°C beforehand) onto the glass slides. The gel films formed on the surfaces were left to cool to room temperature. The samples were stored in a desiccator overnight.

1.2.2.3. Gel formation in vials

The modified glass vials were heated on a hot plate . 90 µl of the hot (60°C) gelator stock solution was pipetted onto the modified vials using a micropipette. This was immediately followed by the addition of 210 µl of water (also preheated to 60°C). The formed gels were left to cool to room temperature. Gel bits were gently removed out of the vial and placed on a microspore slide. The samples were stored in a desiccator overnight.

1.3. Instruments

1.3.1. Water Contact Angle Measurement

The water contact angle (WCA) measurement for the hydroxyl- and phenyl- glass surfaces were performed on a CAM 200 Optical Contact Angle Meter KSV Instrument LTD using CAM200 software. Water droplets were placed on the surfaces and 20 images were taken in 1 sec intervals. Each image was fitted to the circle model and the resulting right and left contact angles were averaged out. At least three measurements were taken on each sample. The WCA measurements are presented as average ± standard deviation (SD).

1.3.2. ToF-SIMS analysis

ToF-SIMS analysis was performed using a ION-TOF TOF-SIMS IV instrument (Münster, Germany). 3 mm x 3 mm raster scans (with 256 x 256 pixels) were obtained using 25 keV Bi_3^+ primary ions with charge compensation. The data was analysed with Surfacelab 6. Positive ion mass spectra were calibrated with m/z 1 (H^+), 15 (CH_3^+), 29 ($C_2H_5^+$), 43 ($C_3H_7^+$) and 57 ($C_4H_9^+$). The analysed areas were divided into four 1.5 mm x 1.5 mm quadrants. For each quadrant, the area under the curve for ions of interest ($C_4H_3^+$ and $C_5H_3^+$) was determined and normalized to the total ion intensities (Table 1).

Table 1: Intensities of ions characteristic for the phenyl group measured on both the OH- and the Ph-surface.

	Normalised Area			
	$C_4H_3^+$		$C_5H_3^+$	
R.O.I.	Ph- surface	OH- surface	Ph- surface	OH- surface
1	0.0219	0.0065	0.0068	0.0032
2	0.0216	0.007	0.007	0.0034
3	0.0215	0.0073	0.007	0.0036
4	0.0219	0.0076	0.007	0.0038
mean ± SD	0.0217±0.0002	0.0071±0.0004	0.0070±0.0001	0.0035±0.0002

1.3.3. AFM imaging

The modified cover glass with and without gels were imaged using a Bruker AFM Probe D300 atomic force microscope in tapping mode (75 kHz, spring constant 3 N/m, cantilever thickness: 3µm, scan rate: 0.5 Hz, target amplitude: 3.0 V). Each image consists of 512 line scans. At least one AFM image was obtained from each of the three repeat samples that were prepared for each gelator/surface combination.

1.3.4. AFM nanoindentation

The mechanical properties were measured on an MFP3D Asylum Research Atomic Force Microscope using contact mode and a trigger point of 0.2 mV. A Multi75 Al probe was used with glass bead of a radius of 12.5 µm attached to the cantilever. The spring constant k was 4.322 N/m and the cantilever resonant frequency 75 kHz. The sensitivity was calculated to be 113 m/V using the InVOLS method where plain clean glass was used as a substrate. The gels were left to dry in a desiccator and were measured within 3 days. For each of the two different surfaces three samples were prepared and five force maps of 16x16 force curves were acquired on a 25x25 µm area. The force curves were then fitted according to the JKR model and the Young's modulus was calculated using the Asylum Research software (version 13). The values for each sample were batch exported to generate three histograms for each type of surface. Young's modulus values of less than 10 MPa were results of bad curve fits as determined individually and thus excluded from the datasets.

The fitting of each histogram was performed using Matlab R2014a and the goodness of fit was tested using the Kolmogorov – Smirnov test function kstest (level of significance 1%). All six datasets fitted the gamma distribution. To compare the Young's modulus distributions of the datasets, the skewness (α) of the fitted gamma distributions was used and a two-tailed student's unpaired t-test (p-value < 0.10) was performed on the datasets (n=3) and the data are presented in Table 2.

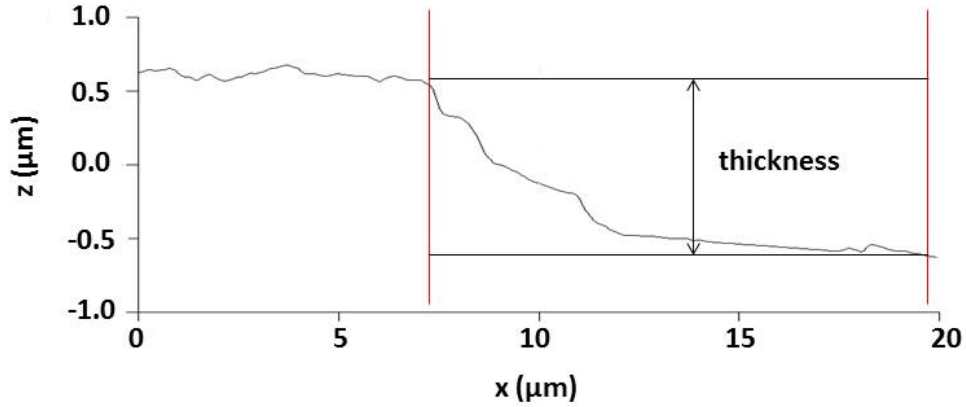
Table 2: Skewness values (α) for the gamma distributions fitted to the Young's modulus values obtained from C14-cytidine gel films.

	skewness	
sample	**OH- surface**	**Ph- surface**
1	1.72	1.4
2	1.46	1.44
3	1.63	1.31
mean ± SD	1.60 ± 0.11	1.38 ± 0.05

2. Supporting data

2.1. Gel thickness measurements

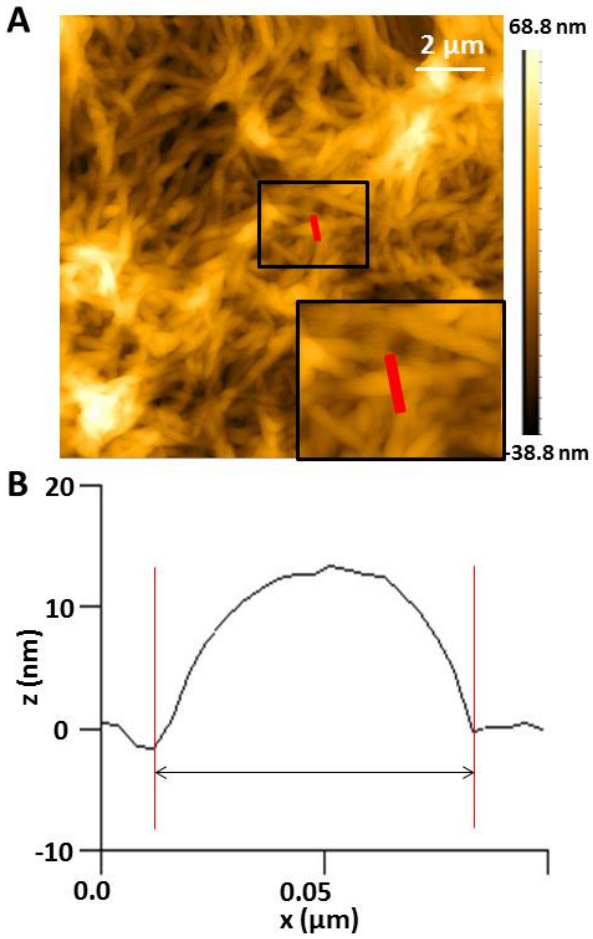
The gel thickness was assessed using Atomic Force Microscopy in tapping mode (cantilever resonant frequency of 75 kHz, spring constant of 3 N/m, cantilever thickness of 3µm and scan rate 0.1 Hz) assessed after scratching the gel in the areas where the force maps were generated with a 21 G needle. Gwyddion 2.41 software was used to analyse the data. On each area a strip with a width of 128 pixels was selected to generate an average line profile. The distance between the cursors was used as the gel height and the measurements were averaged out to determine the thickness of the material (s. fig. 1). The gel thickness was estimated from nine areas and is displayed as mean ± standard deviation (SD).



S. Fig. 1: Typical line profile of scratched gel films obtained by AFM to measure the thickness of the gel film. The vertical axis (z) represents the height (µm) of the sample and the horizontal axis (x) the horizontal distance (µm).

2.2. Determination of Fibre Diameter

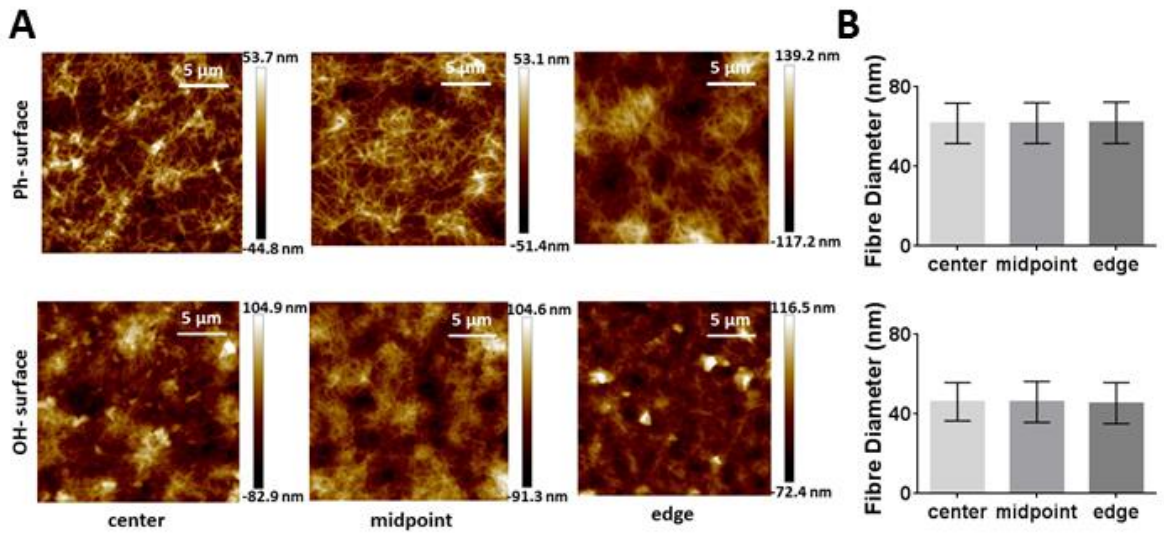
The fibre diameter was estimated using Nanoscope Analysis 1.5 software by measuring the fibre width presented in Figure 3 in the main document. Using a flattened by the software image of 2 x 2 µm, a fibre on the gel surface was selected and the cross section was plotted by the software. The cursors were placed as indicated in S. Fig. 2 and the distance between the two cursors was measured by the software and reported as fibre diameter. One AFM image was obtained from each of the three repeat samples that were prepared for each gelator/surface combination on the coverslips. 20 fibres were measured for each image. Six images were obtained for each condition for the gels in vials and five fibres were measured on each of them.



S. Fig. 2: Procedure for the determination of the fibre diameters. Lines were drawn across fibres on the AFM images (A) to generate line profiles across the fibre widths (B). The fibre edges were determined and the distance between the fibre endpoints was measured to obtain the fibre diameter. The inset in the image (A) is a magnification of the area framed in black. In the line profil (B) the vertical axis (z) represents the height of the sample and the horizontal axis (x) the horizontal distance.

2.3. Gel homogeneity

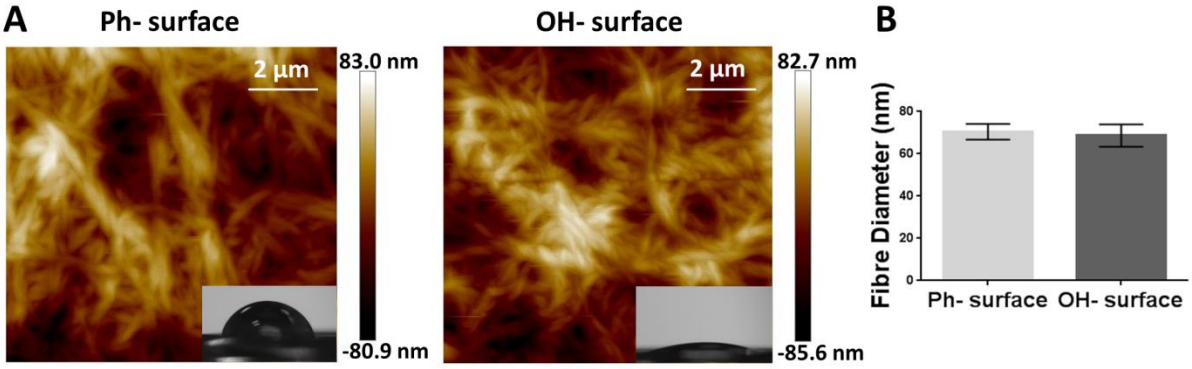
To determine if the gel fibres are uniform across the whole sample area, AFM images were taken at the edge, the centre and middle between the edge and the centre (midpoint) of the sample (S. Fig. 3). The width of 20 gel fibres per sample was measured to determine if fibre diameters vary across the sample. Statistical analysis (One way ANOVA, p-value < 0.05) showed no significant difference.



S. Fig. 3: AFM images (A) and fibre diameters (B) of gel films measured in the centre, at the midpoint and the edge of the gel films.

2.4 Fibre diameters of bulk gel fibres

To obtain an indication as to whether surface effects on self-assembly extend into the bulk of the gels, the diameter of self-assembled fibres from gels prepared in glass vials was measured. Surface modification of the vials was qualitatively confirmed by WCA (insets in S. Fig 4A) but exact WCA measurements on the vials were not possible due to the non-planar geometry of the vials. Gel samples were imaged using AFM (S. Fig. 4A) and fibre diameter were measured. Statistical analysis (One way ANOVA, p-value < 0.05) showed no significant difference.



S. Fig. 4: (A) AFM images of fibres from bulk gels prepared in vials and images of water droplets on the vials (insets in A) displaying either phenyl (Ph) or hydroxyl (OH) groups. (B) Measured diameters of fibres from the bulk gel (n=30).