Supplementary Information

Protein Nanosheet Mechanics Controls Cell Adhesion and Expansion on Low-Viscosity Liquids

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METHODS

Generation of liquid-liquid interfaces for cell culture. 24 well plates were plasma oxidized using a plasma coater (Diener, 100 % intensity) for 10 minutes. 500 µL ethanol (VWR chemicals), 10 µL trimethylamine (Sigma-Aldrich) and 10 µL of the desired silane (triethoxy(octyl)silane (Sigma-Aldrich) to prepare interfaces with liquid PDMS or trichloro (1H, 1H, 2H, 2H-perfluorooctyl) silane (Sigma-Aldrich) for the fluorinated oil) were added into each well. Ethanol was added in between wells to slow down evaporation and parafilm was used to seal the well plate cover. After incubating for 24 h, the wells were washed in a sterile environment with ethanol (twice) and ddH_2O (three times). 500 µL fluorinated oil (Novec 7500, ACOTA) with fluorinated surfactant (2,3,4,5,6-pentafluorobenzoyl chloride, Sigma-Aldrich) at final concentrations of 10, 1, 0.1, 0.01, 0.005, 0.001 and 0 mg/mL was added in the fluorophilic (or oleophilic in the case of PDMS interfaces) 24 well plate to form the bottom liquid layer. Conditioning of the surface was carried out with bovine serum albumin (BSA, 1 mg/mL, Sigma-Aldrich), collagen (type I, 20 µg/mL, Corning) or culture medium (supplemented with foetal bovine serum, 10 %, Labtech) solutions and incubated for 20 min. To wash out these protein solutions, dilutions with sterile PBS (Sigma-Aldrich, 3 times) was carried out, followed by dilution with growth medium.

Generation of PDMS droplet substrates for cell culture. Thin glass slides (25 × 60 mm, VWR) were plasma oxidized for 10 minutes and placed into a staining jar. 100µL triethoxy(octyl)silane (Sigma-Aldrich), 100µL triethylamine (Sigma-Aldrich) and toluene (Sigma-Aldrich, 50 mL) were added to the jar. The jar was covered and sealed with parafilm and left in a fumehood overnight. The resulting hydrophobic thin glass slides were cut into chips (1 x 1 cm) and placed into a 24 well plate. After sterilisation with 70 % ethanol, the wells were washed (twice) and filled with 2 mL PBS (Sigma-Aldrich). 100 µL of liquid PDMS droplets (with viscosities of 10, 50, 1000 , 3500 (Sigma-Aldrich) and 5000 cst, all from ABCR unless specified) with or without surfactant (octanoyl chloride) at 0.5 mg/mL were added on top of the glass slide, resulting in a PDMS droplet that covered approximately 75 % of the surface. The PBS contained within the wells was diluted with growth medium twice. For the deposition of BSA, a 10 µL BSA solution (100 mg/mL) was added into PBS, to make a final concentration of 1 mg/mL, and incubated for 1 h.

Generation of fluorinated oil droplet substrates for cell culture. Thin glass slides (25 × 60 mm, VWR) were plasma oxidized for 10 minutes and placed into a staining jar. Toluene (1 mL, Sigma-Aldrich) and 30 µL trichloro (1H, 1H, 2H, 2H-perfluorooctyl) silane (Sigma-Aldrich) were added in a glass vial. The staining jar containing the glass slides and the glass vial with the silane solution were placed into a desiccator under vacuum for 5 min and then left under reduced atmosphere but sealed overnight. The fluorinated glass slides were cut into chips (1 x 1 cm) and placed into a 24 well plate. After sterilisation with 70 % ethanol, the wells were washed (twice) and then filled with 2 mL PBS (pH 7.4). 100 µL droplets of fluorinated oil (Novec 7500) with fluorinated surfactant (2,3,4,5,6-Pentafluorobenzoyl chloride) at specified concentrations (see specific experiments, but often 0.01 mg/mL) were deposited on top of the glass slide and formed a fluorinated oil droplet spreading over the entire substrate. 30 µL oil was removed by micropipette to form a flatter and more stable oil droplet. For the deposition of BSA, a 10 µL BSA solution (100 mg/mL) was added into PBS, to make a final concentration of 1 mg/mL, and incubated for 1 h.

Generation of emulsions for characterisation. 1 mL fluorinated oil (Novec 7500, ACOTA) containing the fluorinated surfactant 2,3,4,5,6-Pentafluorobenzoyl chloride at final concentrations of 10, 1, 0.1, 0.01, 0.005, 0.001 and 0 mg/mL and 2 mL BSA (1 mg/ml solution in PBS) were added into a 15 ml centrifuge tube. The tube was vigorously shaken manually to mix and generate the emulsion and subsequently left to incubate at room temperature for 1 h. The top liquid phase, above the settled emulsion was aspirated and replaced with PBS 6 times and deionised water twice. Droplets of emulsion were then transferred to silicon substrates (silicon wafer, Pi-Kem Ltd.) and left to dry in air for SEM, XPS and AFM analysis (see below). Samples analysed by XPS and AFM were further washed with ethanol before drying.

Hoechst staining and LIVE/DEAD cell viability assay. Cell proliferation was assessed via Hoechst staining. Cells were incubated in DMEM containing 5 µL Hoechst 33342 (1 mg/mL, Thermofisher Scientific) for 30 min before imaging by epifluorescence microscopy (see below). Viability of HaCaT cells on fluorinated oil interfaces was quantified by LIVE/DEAD viability/cytotoxicity assay using a kit supplied by Thermofisher Scientific. In brief, HaCaT cells were incubated in DMEM with 2 µM Calcein AM and 4 µM Ethidium homodimer for 30 min. Stained cells were imaged using a Leica DMI4000 fluorescence microscopy (see below). The percentage of viable cells was calculated by counting the number of green (live) cells and dividing by the total number of cells (including dead cells).

Labelling of FBS with Alexa Fluor 594 and adsorption to interfaces for imaging. Emulsions were generated with fluorinated Novec 7500 oil, in the presence of 0.01 mg/mL PFBC and BSA (1 mg/mL) solution in a 15 mL centrifuge tube. 50 µL emulsions were transferred in one well (24 well plates). For the adsorption of labelled FBS, a 50 µL labelled FBS solution (1mg/mL, prepared with Alexa Fluor 594 protein labelling kit) was added to this well and incubated at room temperature for 1 h. The FBS solution was then diluted with PBS (pH 7.4) 6 times prior to imaging of the remaining droplets via epifluorescence microscopy.

Immuno-fluorescence microscopy and data analysis. Fluorescence microscopy images were acquired with a Leica DMI4000B fluorescence microscopy (CTR4000 lamp; 63 × 1.25 NA, oil lens; 10 × 0.3 NA lens; 2.5 x 0.07 NA lens; DFC300FX camera). To determine cell densities per mm^2, cell counting was carried out by thresholding and watershedding nuclei images in ImageJ. In the case of cell clumps, for which this protocol did not allow the isolation of individual nuclei, cells were counted manually. To determine adhesion cell areas, images were analyzed by thresholding and watershedding fluorescence images of the cytoskeleton (phalloidin stained). The area of cell clusters was removed when analysing results.

HaCaT keratinocyte cell line culture and seeding. Human keratinocyte HaCaT cells were cultured in DMEM (Thermofisher Scientific) containing 10 % foetal bovine serum (FBS, Labtech), 1 % L-Glutamine (200 mM) and 1 % Penicillin-Streptomycin (5,000 U/mL). For proliferation assays, HaCaT cells were harvested with trypsin (0.25 %) and versene solutions (Thermofisher Scientific, 0.2 g/L EDTA Na_4 in Phosphate Buffered Saline) in a ratio of 1/9, centrifuged, counted and resuspended in DMEM at the desired density before seeding onto substrates (conditioned as stated above, in a 24-well plate) at a density of 2,000 per well (1,000 cells per cm^2). Cells were left to adhere and proliferate in an incubator (37 °C and 5 % CO2) for different time points, prior to staining and imaging. For cell spreading assays HaCaT cells were harvested and seeded onto fluorinated droplets at a density of 25,000 per well (13,000 per cm^2). For passaging, cells were reseeded in a T75 at a density of 250k cells per flask.

Interfacial rheology. Rheological measurements were carried out on a hybrid rheometer (DHR-3) from TA Instruments fitted with a double wall ring (DWR) geometry and a Delrin trough with a circular channel. The double wall ring used for this geometry has a radius of 34.5 mm and the thickness of the Platinum–Iridium wire is 1 mm. The diamond-shaped cross-section of the geometry's ring provides the capability to pin directly onto the interface between two liquids and measure the interface properties without complicated sub-phase correction. 19 mL of the fluorinated oil pre-mixed with surfactant were placed in the Delrin trough and the ring was lowered, ensuring contact with the surface, via an axial force procedure. The measuring position was set 500 µm lower than the contact point of the ring with the oil-phase surface. Thereafter, 15 ml of the PBS buffer were carefully syringed on top of the oil phase. Time sweeps were performed at a constant frequency of 0.1 Hz and a temperature of 25 ^{o}C, with a displacement of 1.0 10^{-3} rad to follow the formation of the protein layers at the interface. The concentration of BSA used for all rheology experiments was 1 mg/mL (with respect to aqueous phase volume). Before and after each time sweep, frequency sweeps (with a constant displacement of 1.0 10^{-3} rad) were conducted to examine the frequency-dependant characteristics of the interface whilst amplitude sweeps (with constant frequencies of 0.1 Hz) were carried out to ensure that the chosen displacement was within the linear viscoelastic region.

Colloidal AFM. Indentation testing was performed on the series of oil-water interfaces using the following protocol. A Bruker 8-10 AFM tip was used with a colloidal Si bead attached with a radius of 20 µm (freshly plasma treated). The tip was first calibrated against a Si wafer. Samples were characterised in three different locations with a grid of 10×10 indentations, acquiring 300 curves per sample. This data was then analysed using a MATLAB script. The stiffness of the interfaces was characterised as a spring constant from the gradient of the Force-Displacement traces.

Scanning electron microscopy. The dried protein-coated droplets deposited on silicon substrates were coated with gold for 30 s before imaged using an FEI Inspect F scanning electron microscopy operated at 10 kV. A spot size of 3.5 and an aperture of 30 mm were used. Experiments were repeated twice and five areas were analysed at different magnifications (140x, 1200x, 8000x and 80000x).

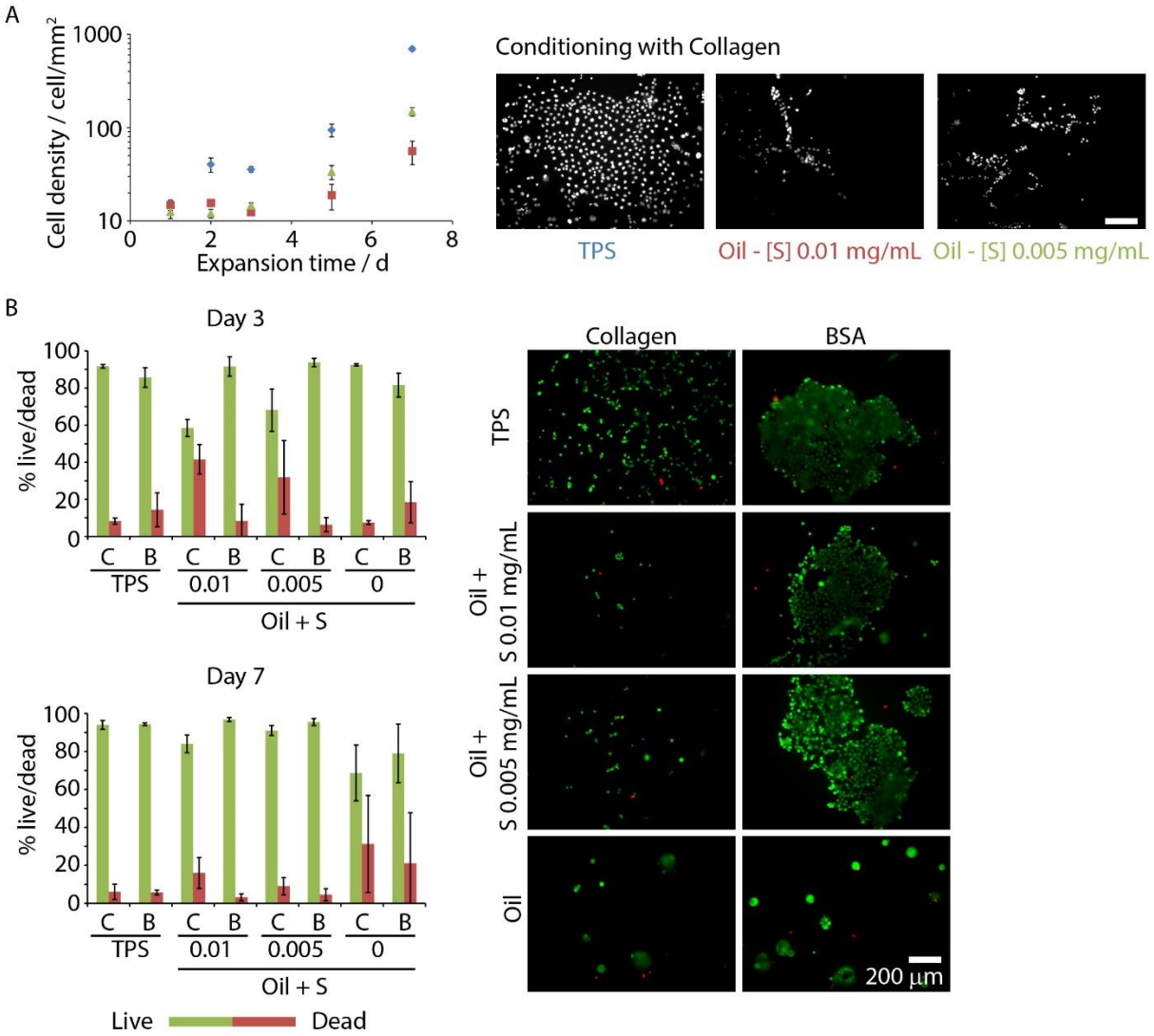
Atomic force microscopy (AFM). The dried protein-coated droplets deposited on a silicon substrate were directly imaged by AFM without further treatment. In order to assess the thickness of the protein layers, the samples were gently scratched in different point with the tip of metal tweezers (Dumont). For the imaging, an AFM (NT-MDT NTEGRA) was used in semi contact mode topography. The surface of the substrate was visualised via an optical microscope to identify the localisation of the scratches. The scans were conducted at a frequency of 1.01 Hz. The areas scanned were of 50 by 50 µm. The probes used were for non-contact mode from NT-MDT (resonant frequency between 87- 230 kHz and force constant 1.45- 15.1 N/m). Pictures were corrected via the software subtracting a 1st order curve. The profiles across the scratch were then analysed and the differences in height were measured via the software, affording a direct measurement of the thickness of the dried protein layer (after correcting by a factor of 2 as the drying of a single droplet results in the deposition of two protein layers). Experiments were repeated twice and five areas were analysed for each condition, taking between 5 and 10 measurements for each scan.

X-ray photoelectron spectroscopy. XPS was carried out using a Kratos Axis Ultra DLD electron spectrometer with a monochromated Al Kα source (1486.6 eV) operated at 150 W. A pass energy of

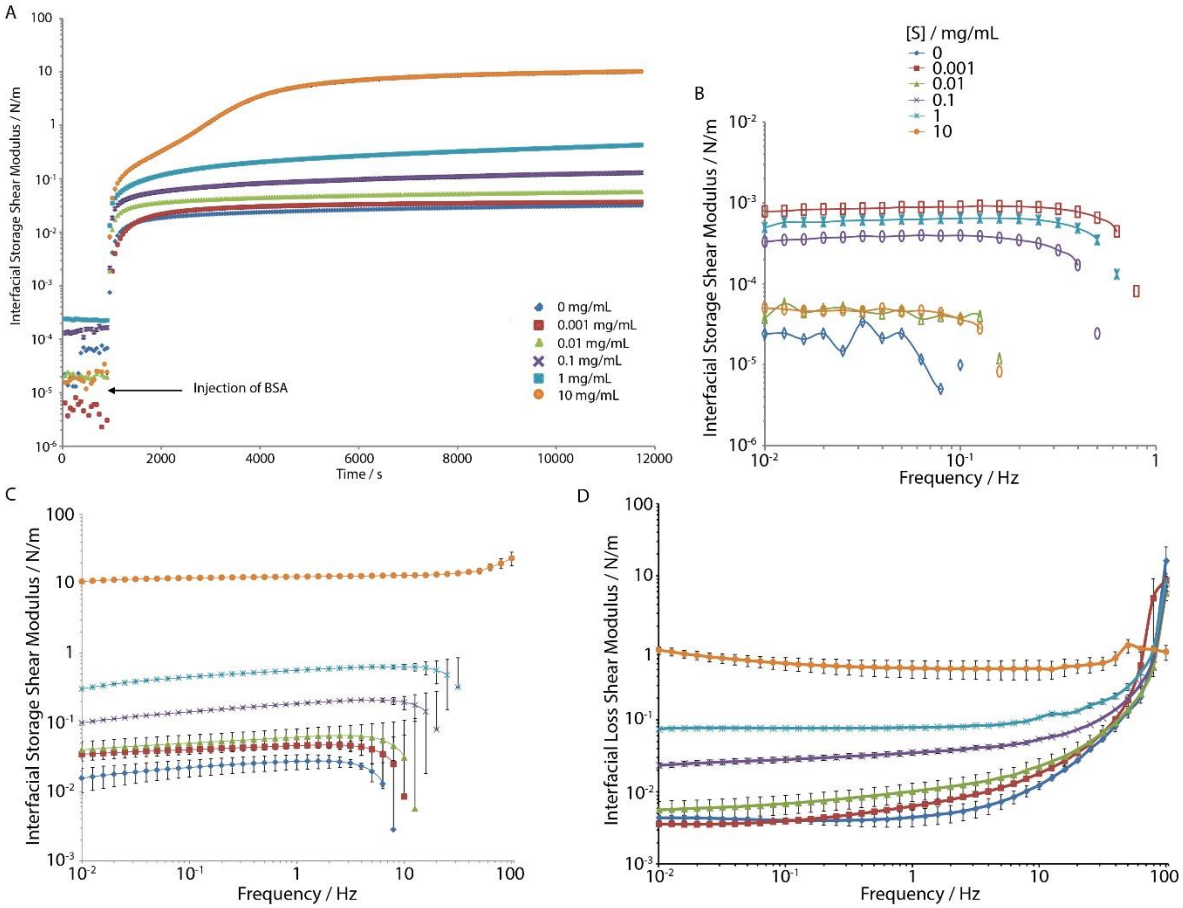
160 eV and a step size of 1 eV were used for survey spectra. For high energy resolution spectra of regions, a pass energy of 20 eV and a step size of 0.1 eV were used. The spectrometer charge neutralising system was used to compensate sample charging and the binding scale was referenced to the aliphatic component of C 1s spectra at 285.0 eV. The concentrations obtained (error less than ± 10 %) are reported as the percentage of that particular atom species (atomic %) at the surface of the sample (< 10 nm analysis depth) without any correction. The analysis area (0.3×0.7 mm^2), the angle of incidence and the beam intensity were kept constant for all measurements. To determine the functionalisation level of BSA macromolecules with PFBC surfactant, atomic % reported in the literature were used (62.6 % for C 1s, 14.4 % for N 1s and 23.0 for O 1s)[1] and a molecular weight of 66 kDa was used in the calculations to determine a standard curve (and equation) predicting the evolution of the F 1s atomic % as a function of the number of PFBC surfactant tethered. For the functionalisation of PLL, this calculation was directly based on the molar mass of lysine repeat units.

Attenuated Total Reflectance - Fourier Transformed Infrared (ATR-FTIR) Spectroscopy. All ATR-FTIR measurements were performed on a Brucker Tensor 27 infrared spectrometer equipped with an MCT detector, cooled with liquid N_2. 2 ml fluorinated oil (Novec 7500, ACOTA) with fluorinated surfactant (2,3,4,5,6-Pentafluorobenzoyl chloride) at final concentrations of 10 mg/mL and 4 ml BSA 1 mg/mL solution were added into a glass vial. The vial was vigorously shaken manually to mix and form the emulsion and subsequently left to incubate at room temperature for 1 h. To wash the emulsion, the top liquid phase, above the settled emulsion was aspirated and replaced with PBS 6 times and deionised water twice. The glass vials was then left overnight under vacuum resulting in a dry white residue. One sample of this dry material was washed further with ethanol to remove any unbound surfactant, whilst one sample was directly analysed by FTIR spectroscopy. The dried residues, 2,3,4,5,6-Pentafluorobenzoyl chloride, and BSA powder were characterised by FTIR spectroscopy.

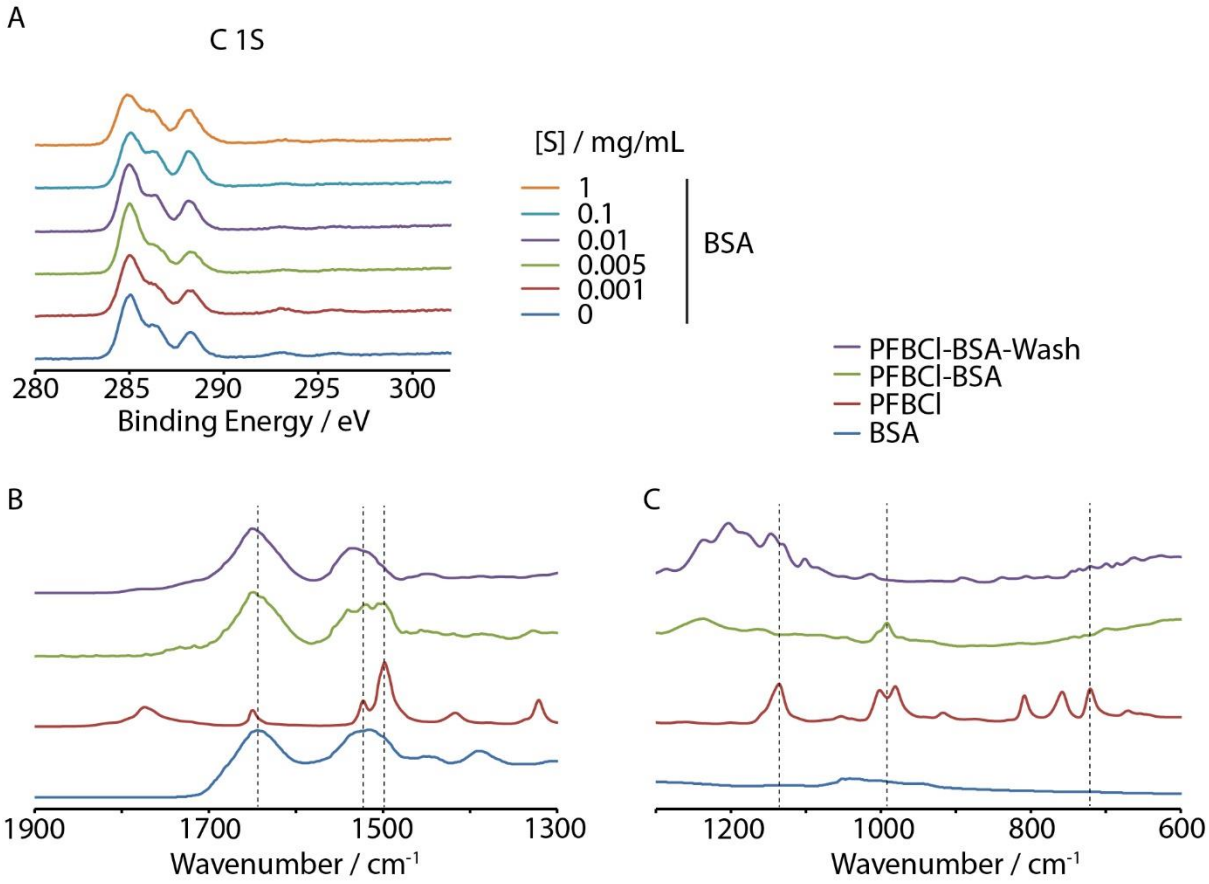
Statistical analysis. Statistical analysis was carried out using Origin 8 through one-way ANOVA with Tukey test for posthoc analysis. Significance was determined by * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ and n.s., non-significant. A full summary of statistical analysis is provided in the supplementary information.



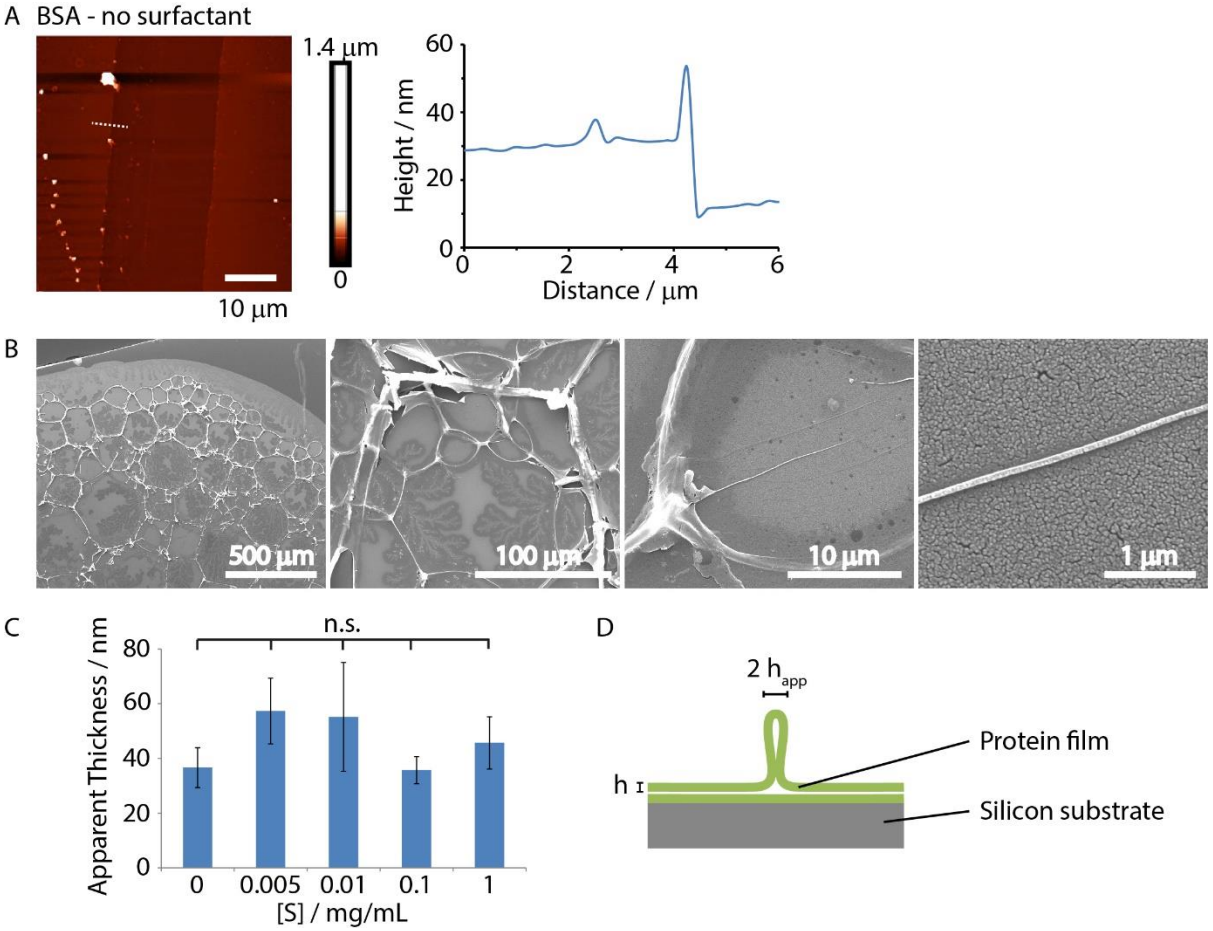
Supplementary Figure S1. Impact of conditioning of the liquid-liquid interface on HaCaT cell proliferation and viability. A. HaCaT proliferation profile on interfaces conditioned with collagen (20 µg/mL; blue diamonds, TPS; red square, Novec 7500 + 0.01 mg/mL PFBC; green triangles, Novec 7500 + 0.005 mg/mL PFBC). B. HaCaT cell viability (live, green; dead, red) when cultured on interfaces conditioned with collagen type I (C) or BSA (B). For oil + PFBC (surfactant, S), the corresponding concentrations of surfactant are stated (in mg/mL). Error bars are s.e.m.; n=3.



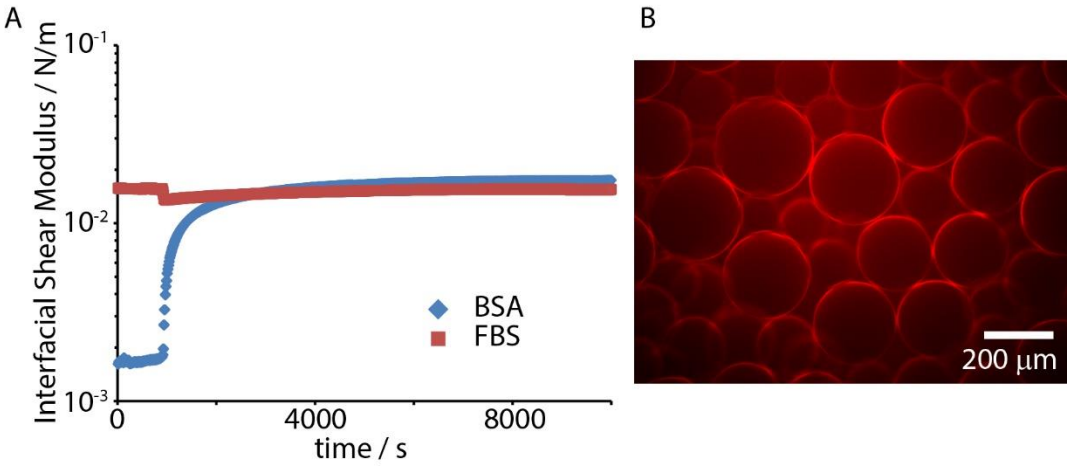
Supplementary Figure S2. Characterisation of mechanical properties of liquid-liquid interfaces via interfacial rheology. A. Time sweep (0.1 Hz, oscillating amplitude of 10^{-3} rad) experiments carried out at different surfactant (PFBC) concentrations (see legend), in the presence of BSA (1 mg/mL). Frequency sweep experiments (oscillating amplitude of 10^{-3} rad) carried out for the characterisation of interfaces generated at different concentrations of PFBC (see legend); B and C are the interfacial storage moduli without and with exposure to 1 mg/mL BSA solutions, respectively; D, interfacial loss shear modulus recorded after exposure to 1 mg/mL BSA solutions.



Supplementary Figure S3. Characterisation of the chemical composition of protein assemblies generated at liquid-liquid interfaces. XPS spectra (C 1S) obtained for dried emulsions generated with Novec 7500 (different concentrations of PFBC surfactant [S]) and BSA (1 mg/mL). B and C. Zooms of FTIR spectra (see Figure 3) obtained for pristine BSA, the surfactant PFBC, protein films generated in emulsions (10 mg/mL PFBC and 1 mg/mL BSA) before (PFBCl-BSA) and after (PFBCl-BSA-wash) washing with ethanol.



Supplementary Figure S4. Characterisation of the thickness of protein layers generated at liquid-liquid interfaces. A. AFM characterisation (height image and profile) of oil droplets (no surfactant and BSA at 1 mg/mL), dried onto silicon substrates. B. SEM images of oil droplets (no surfactant, BSA = 1 mg/mL), dried onto silicon substrates. C. Characterisation of the thickness of protein layers from SEM images. Error bars are s.e.m.; n=10 from two separate experiments. D. Schematic representation of a protein layer wrinkle.



Supplementary Figure S5. Conditioning of BSA nanosheets. A. Time sweep (0.1 Hz, oscillating amplitude of 10^{-3} rad) experiments carried out at a concentration of PFBC as 0.01 mg/mL, in the presence of BSA (blue diamonds; 1 mg/mL) and FBS (red squares; 10%, following a first adsorption of BSA 1 mg/mL). Little further increase in interfacial shear modulus is observed upon incubation of BSA interfaces in serum solutions. B. To establish whether protein adsorption occurs at the surface of BSA-coated oil, emulsions generated with fluorinated oil in the presence of 0.01 mg/mL PFBC and BSA solution were subsequently incubated with 10% FBS (labelled with Alexa Fluor 594). Scale bars are 200 µm.

Supplementary Table S1. Summary of statistical data obtained for HaCaT cells proliferation on a fluorinated oil (Novec 7500, 0.77 cSt) containing the surfactant pentafluorobenzoyl chloride at different concentrations (see Figure 1A).

	MeanDiff	Prob	
S0 TPS	-117.435	0.00473	**
S0.0005 TPS	-96.9738	0.06371	n.s.
S0.0005 S0	20.4607	0.99964	n.s.
S0.001 TPS	-52.6649	0.72274	n.s.
S0.001 S0	64.76965	0.45635	n.s.
S0.001 S0.0005	44.30894	0.92578	n.s.
S0.005 TPS	-26.2873	0.9919	n.s.
S0.005 S0	91.14724	0.04455	*
S0.005 S0.0005	70.68654	0.34017	n.s.
S0.005 S0.001	26.3776	0.99663	n.s.
S0.01 TPS	2.8907	1	n.s.
S0.01 S0	120.3252	0.00368	**
S0.01 S0.0005	99.8645	0.05171	n.s.
S0.01 S0.001	55.55556	0.65995	n.s.
S0.01 S0.005	29.17796	0.98217	n.s.
S0.05 TPS	-111.021	0.00826	**
S0.05 S0	6.41373	1	n.s.
S0.05 S0.0005	-14.047	0.99999	n.s.
S0.05 S0.001	-58.3559	0.59732	n.s.
S0.05 S0.005	-84.7335	0.0747	n.s.
S0.05 S0.01	-113.911	0.00643	**
S0.1 TPS	-120.145	0.00374	**
S0.1 S0	-2.71003	1	n.s.
S0.1 S0.0005	-23.1707	0.9989	n.s.
S0.1 S0.001	-67.4797	0.4009	n.s.
S0.1 S0.005	-93.8573	0.03562	*
S0.1 S0.01	-123.035	0.00291	**
S0.1 S0.05	-9.12376	1	n.s.
S0.5 TPS	-124.119	0.00265	**
S0.5 S0	-6.68473	1	n.s.
S0.5 S0.0005	-27.1454	0.99572	n.s.
S0.5 S0.001	-71.4544	0.32651	n.s.
S0.5 S0.005	-97.832	0.02554	*
S0.5 S0.01	-127.01	0.00206	**
S0.5 S0.05	-13.0985	0.99999	n.s.
S0.5 S0.1	-3.97471	1	n.s.
S1 TPS	-122.584	0.0093	**
S1 S0	-5.14905	1	n.s.
S1 S0.0005	-25.6098	0.99881	n.s.
S1 S0.001	-69.9187	0.47638	n.s.
S1 S0.005	-96.2963	0.06687	n.s.

S1 S0.01	-125.474	0.00744	**
S1 S0.05	-11.5628	1	n.s.
S1 S0.1	-2.43902	1	n.s.
S1 S0.5	1.53568	1	n.s.
S5 TPS	-121.635	0.01001	*
S5 S0	-4.20054	1	n.s.
S5 S0.0005	-24.6613	0.99915	n.s.
S5 S0.001	-68.9702	0.49505	n.s.
S5 S0.005	-95.3478	0.07153	n.s.
S5 S0.01	-124.526	0.008	**
S5 S0.05	-10.6143	1	n.s.
S5 S0.1	-1.49051	1	n.s.
S5 S0.5	2.48419	1	n.s.
S5 S1	0.94851	1	n.s.
S10 TPS	-123.397	0.00873	**
S10 S0	-5.96206	1	n.s.
S10 S0.0005	-26.4228	0.99843	n.s.
S10 S0.001	-70.7317	0.46058	n.s.
S10 S0.005	-97.1093	0.06309	n.s.
S10 S0.01	-126.287	0.00698	**
S10 S0.05	-12.3758	1	n.s.
S10 S0.1	-3.25203	1	n.s.
S10 S0.5	0.72267	1	n.s.
S10 S1	-0.81301	1	n.s.
S10 S5	-1.76152	1	n.s.

*TPS, tissue culture polystyrene; S0/0.0005/0.001/0.005/0.01/0.05/0.1/0.5/1/5/10 describe the concentration (mg/ml) of the surfactant pentafluorobenzoyl chloride used in fluorinated oil (Novec 7500, 0.77 cSt). n.s., non-significant; *, P < 0.05; ** P < 0.01; *** P < 0.001.*

Supplementary Table S2. Summary of statistical data obtained for HaCaT cells proliferation profile on interfaces conditioned with BSA (see Figure 2A).

		MeanDiff	**Prob**	
	S0.01 TPS	2.71003	0.24996	n.s.
Day 1	S0.005 TPS	6.50407	0.01202	*
	S0.005 S0.01	3.79404	0.10079	n.s.
		MeanDiff	Prob	
	S0.01 TPS	3.88437	0.77075	n.s.
Day 2	S0.005 TPS	25.47425	0.00871	**
	S0.005 S0.01	21.58988	0.01858	*
		MeanDiff	Prob	
	S0.01 TPS	4.33604	0.65489	n.s.
Day 3	S0.005 TPS	34.86902	8.17E-04	***
	S0.005 S0.01	30.53297	0.00167	**
		MeanDiff	Prob	
	S0.01 TPS	-4.06504	0.73844	n.s.
Day 5	S0.005 TPS	4.15537	0.72897	n.s.
	S0.005 S0.01	8.22042	0.3394	n.s.
		MeanDiff	Prob	
	S0.01 TPS	-15.0858	0.96399	n.s.
Day 7	S0.005 TPS	447.9675	6.23E-04	***
	S0.005 S0.01	463.0533	5.19E-04	***

*TPS, tissue culture polystyrene; S0.005/0.01 describe the concentration (mg/ml) of the surfactant pentafluorobenzoyl chloride used in a fluorinated oil (Novec 7500, 0.77 cSt). n.s., non-significant; *, P < 0.05; ** P < 0.01; *** P < 0.001.*

Supplementary Table S3. Summary of statistical data obtained for HaCaT cells proliferation profile on silicone oils with viscosities in the range of 10-5000 cSt (see Figure 2B).

Day 7	MeanDiff	Prob	
No S 10 cSt - TPS	-705.691	1.11E-06	***
0.5 S 10 cSt - TPS	-388.347	0.00235	**
0.5 S 10 cSt - No S 10 cSt	317.3442	0.01554	*
No S 50 cSt - TPS	-704.878	1.12E-06	***
No S 50 cSt - No S 10 cSt	0.81301	1	n.s.
No S 50 cSt - 0.5 S 10 cSt	-316.531	0.01588	*
0.5 S 50 cSt - TPS	-359.801	0.00503	**
0.5 S 50 cSt - No S 10 cSt	345.8898	0.00729	**
0.5 S 50 cSt - 0.5 S 10 cSt	28.54562	0.99998	n.s.
0.5 S 50 cSt - No S 50 cSt	345.0768	0.00744	**
No S 1000 cSt - TPS	-705.962	1.10E-06	***
No S 1000 cSt - No S 10 cSt	-0.271	1	n.s.

No S 1000 cSt - 0.5 S 10 cSt	-317.615	0.01543	*
No S 1000 cSt - No S 50 cSt	-1.08401	1	n.s.
No S 1000 cSt - 0.5 S 50 cSt	-346.161	0.00723	**
0.5 S 1000 cSt - TPS	-224.932	0.15462	n.s.
0.5 S 1000 cSt - No S 10 cSt	480.7588	2.11E-04	***
0.5 S 1000 cSt - 0.5 S 10 cSt	163.4146	0.50006	n.s.
0.5 S 1000 cSt - No S 50 cSt	479.9458	2.15E-04	***
0.5 S 1000 cSt - 0.5 S 50 cSt	134.869	0.71889	n.s.
0.5 S 1000 cSt - No S 1000 cSt	481.0298	2.09E-04	***
No S 5000 cSt - TPS	-706.143	1.09E-06	***
No S 5000 cSt - No S 10 cSt	-0.45167	1	n.s.
No S 5000 cSt - 0.5 S 10 cSt	-317.796	0.01536	*
No S 5000 cSt - No S 50 cSt	-1.26468	1	n.s.
No S 5000 cSt - 0.5 S 50 cSt	-346.341	0.0072	**
No S 5000 cSt - No S 1000 cSt	-0.18067	1	n.s.
No S 5000 cSt - 0.5 S 1000 cSt	-481.21	2.08E-04	***
0.5 S 5000 cSt - TPS	-425.655	8.75E-04	***
0.5 S 5000 cSt - No S 10 cSt	280.0361	0.04098	*
0.5 S 5000 cSt - 0.5 S 10 cSt	-37.308	0.99988	n.s.
0.5 S 5000 cSt - No S 50 cSt	279.2231	0.04184	*
0.5 S 5000 cSt - 0.5 S 50 cSt	-65.8537	0.99326	n.s.
0.5 S 5000 cSt - No S 1000 cSt	280.3071	0.0407	*
0.5 S 5000 cSt - 0.5 S 1000 cSt	-200.723	0.25847	n.s.
0.5 S 5000 cSt - No S 5000 cSt	280.4878	0.04051	*

*TPS, tissue culture polystyrene; the surfactant octanoyl chloride was used at a concentration of 0.05 mg/mL (0.5S) with 10, 50, 1000 and 5000 cSt PDMS oils; the protein deposited was BSA, 1 mg/mL. Experiments carried out in the absence of surfactant are denoted as "No S". n.s., non-significant; *, P < 0.05; ** P < 0.01; *** P < 0.001.*

Supplementary Table S4. Summary of statistical data obtained for AFM quantification of thickness of oil droplets (see Figure 4C).

		MeanDiff	Prob	
BSA-PFBC s0.01	BSA	3.78546	0.21598	n.s.

*s0.01 describes the concentration (mg/mL) of the surfactant pentafluorobenzoyl chloride (PFBC) used in a fluorinated oil (Novec 7500, 0.77 cSt).BSA, Bovine serum albumin 1mg/mL. n.s., non-significant; *, P < 0.05; ** P < 0.01; *** P < 0.001.*

Supplementary Table S5. Summary of statistical data obtained for film stiffness characterised by interfacial AFM (see Figure 5B).

	MeanDiff	**Prob**	
Oil+BSA Oil	-0.01045	4.44E-16	***
Oil+[0.01]s Oil	-0.00655	4.44E-16	***
Oil+[0.01]s Oil+BSA	0.00389	1.60E-06	***
Oil+[0.01]s+BSA Oil	-0.01199	4.44E-16	***
Oil+[0.01]s+BSA Oil+BSA	-0.00155	0.379	n.s.
Oil+[0.01]s+BSA Oil+[0.01]s	-0.00544	9.57E-13	***
Oil+[0.005]s+BSA Oil	-0.01236	4.44E-16	***
Oil+[0.005]s+BSA Oil+BSA	-0.00192	0.131	n.s.
Oil+[0.005]s+BSA Oil+[0.01]s	-0.00581	1.59E-14	***
Oil+[0.005]s+BSA Oil+[0.01]s+BSA	-3.71E-04	1	n.s.
Oil+[0.001]s+BSA Oil	-0.0113	4.44E-16	***
Oil+[0.001]s+BSA Oil+BSA	-8.50E-04	0.936	n.s.
Oil+[0.001]s+BSA Oil+[0.01]s	-0.00474	1.07E-09	***
Oil+[0.001]s+BSA Oil+[0.01]s+BSA	6.96E-04	0.979	n.s.
Oil+[0.001]s+BSA Oil+[0.005]s+BSA	0.00107	0.814	n.s.

*BSA, Bovine serum Albumin, [0.01]s describe the concentration (mg/ml) of the surfactant pentafluorobenzoyl chloride used in the fluorinated oil, BSA always used at 1mg/m, n.s., non-significant; *, P < 0.05; ** P < 0.01; *** P < 0.001.*

Supplementary Table S6. Summary of statistical data obtained for HaCaT cells proliferation profile on interfaces conditioned with collagen (see Supplementary Figure S1A).

		MeanDiff	Prob	
	S0.01 TPS	-0.81301	0.93741	n.s.
Day 1	S0.005 TPS	-3.1617	0.42675	n.s.
	S0.005 S0.01	-2.34869	0.6065	n.s.
		MeanDiff	**Prob**	
	S0.01 TPS	-24.7516	0.00522	**
Day 2	S0.005 TPS	-28.2746	0.00266	**
	S0.005 S0.01	-3.52304	0.75639	n.s.
		MeanDiff	**Prob**	
	S0.01 TPS	-23.3062	3.28E-05	***
Day 3	S0.005 TPS	-21.0479	5.92E-05	***
	S0.005 S0.01	2.25836	0.46946	n.s.
		MeanDiff	**Prob**	
	S0.01 TPS	-75.3388	9.94E-04	***
Day 5	S0.005 TPS	-60.7046	0.0031	**
	S0.005 S0.01	14.63415	0.41252	n.s.
		MeanDiff	**Prob**	
	S0.01 TPS	-639.386	6.91E-07	***
Day 7	S0.005 TPS	-546.793	1.81E-06	***
	S0.005 S0.01	92.59259	0.02748	*

*TPS, tissue culture polystyrene; S0.005/0.01 describe the concentration (mg/ml) of the surfactant pentafluorobenzoyl chloride used in a fluorinated oil (Novec 7500, 0.77 cSt). n.s., non-significant; *, P < 0.05; ** P < 0.01; *** P < 0.001.*

Supplementary Table S7. Summary of statistical data obtained for HaCaT cell viability (live; dead) when cultured on interfaces conditioned with collagen type I (C) or BSA (B) (see Supplementary Figure S1B).

Day 3			
live	**MeanDiff**	**Prob**	
TPS-B TPS-C	-6.0498	0.99971	n.s.
S0.01-C TPS-C	-33.2637	0.01723	*
S0.01-C TPS-B	-27.2139	0.08598	n.s.
S0.01-B TPS-C	-0.12881	1	n.s.
S0.01-B TPS-B	5.92099	0.99977	n.s.
S0.01-B S0.01-C	33.13489	0.01786	*
S0.005-C TPS-C	-23.5578	0.20192	n.s.
S0.005-C TPS-B	-17.508	0.58938	n.s.
S0.005-C S0.01-C	9.7059	0.9838	n.s.
S0.005-C S0.01-B	-23.429	0.2076	n.s.
S0.005-B TPS-C	2.01222	1	n.s.
S0.005-B TPS-B	8.06202	0.99628	n.s.
S0.005-B S0.01-C	35.27592	0.00978	**
S0.005-B S0.01-B	2.14103	1	n.s.
S0.005-B S0.005-C	25.57002	0.12806	n.s.
S0-B TPS-C	0.83454	1	n.s.
S0-B TPS-B	6.88434	0.99906	n.s.
S0-B S0.01-C	34.09824	0.01364	*
S0-B S0.01-B	0.96335	1	n.s.
S0-B S0.005-C	24.39234	0.16797	n.s.
S0-B S0.005-B	-1.17768	1	n.s.
S0-C TPS-C	-10.1468	0.97758	n.s.
S0-C TPS-B	-4.09702	0.99999	n.s.
S0-C S0.01-C	23.11687	0.22188	n.s.
S0-C S0.01-B	-10.018	0.97956	n.s.
S0-C S0.005-C	13.41098	0.87026	n.s.
S0-C S0.005-B	-12.1591	0.92568	n.s.
S0-C S0-B	-10.9814	0.96117	n.s.
dead	**MeanDiff**	**Prob**	
TPS-B TPS-C	6.0498	0.99971	n.s.
S0.01-M TPS-C	3.41798	1	n.s.
S0.01-M TPS-B	-2.63182	1	n.s.
S0.01-C TPS-C	33.2637	0.01723	*
S0.01-C TPS-B	27.2139	0.08598	n.s.
S0.01-B TPS-C	0.12881	1	n.s.
S0.01-B TPS-B	-5.92099	0.99977	n.s.
S0.01-B S0.01-C	-33.1349	0.01786	*
S0.005-C TPS-C	23.5578	0.20192	n.s.
S0.005-C TPS-B	17.508	0.58938	n.s.

S0.005-C S0.01-C	-9.7059	0.9838	n.s.
S0.005-C S0.01-B	23.42899	0.2076	n.s.
S0.005-B TPS-C	-2.01222	1	n.s.
S0.005-B TPS-B	-8.06202	0.99628	n.s.
S0.005-B S0.01-C	-35.2759	0.00978	**
S0.005-B S0.01-B	-2.14103	1	n.s.
S0.005-B S0.005-C	-25.57	0.12806	n.s.
S0-B TPS-C	-0.83454	1	n.s.
S0-B TPS-B	-6.88434	0.99906	n.s.
S0-B S0.01-C	-34.0982	0.01364	*
S0-B S0.01-B	-0.96335	1	n.s.
S0-B S0.005-C	-24.3923	0.16797	n.s.
S0-B S0.005-B	1.17768	1	n.s.
S0-C TPS-C	10.14682	0.97758	n.s.
S0-C TPS-B	4.09702	0.99999	n.s.
S0-C S0.01-C	-23.1169	0.22188	n.s.
S0-C S0.01-B	10.01801	0.97956	n.s.
S0-C S0.005-C	-13.411	0.87026	n.s.
S0-C S0.005-B	12.15905	0.92568	n.s.
S0-C S0-B	10.98137	0.96117	n.s.

Day 7

live	**MeanDiff**	**Prob**	
TPS-B TPS-C	0.3828	1	n.s.
S0.01-M TPS-C	2.42813	1	n.s.
S0.01-M TPS-B	2.04534	1	n.s.
S0.01-C TPS-C	-9.95626	0.99441	n.s.
S0.01-C TPS-B	-10.3391	0.99242	n.s.
S0.01-B TPS-C	2.8961	1	n.s.
S0.01-B TPS-B	2.5133	1	n.s.
S0.01-B S0.01-C	12.85236	0.96215	n.s.
S0.005-C TPS-C	-2.91861	1	n.s.
S0.005-C TPS-B	-3.3014	1	n.s.
S0.005-C S0.01-C	7.03766	0.99974	n.s.
S0.005-C S0.01-B	-5.8147	0.99996	n.s.
S0.005-B TPS-C	1.52081	1	n.s.
S0.005-B TPS-B	1.13801	1	n.s.
S0.005-B S0.01-C	11.47708	0.98304	n.s.
S0.005-B S0.01-B	-1.37528	1	n.s.
S0.005-B S0.005-C	4.43942	1	n.s.
S0-B TPS-C	-25.2378	0.30458	n.s.
S0-B TPS-B	-25.6206	0.28567	n.s.
S0-B S0.01-C	-15.2815	0.8898	n.s.
S0-B S0.01-B	-28.1339	0.18159	n.s.
S0-B S0.005-C	-22.3192	0.47248	n.s.
S0-B S0.005-B	-26.7586	0.23425	n.s.

S0-C TPS-C	-14.9994	0.90079	n.s.
S0-C TPS-B	-15.3822	0.88571	n.s.
S0-C S0.01-C	-5.04315	0.99999	n.s.
S0-C S0.01-B	-17.8955	0.75797	n.s.
S0-C S0.005-C	-12.0808	0.97537	n.s.
S0-C S0.005-B	-16.5202	0.83365	n.s.
S0-C S0-B	10.23838	0.99299	n.s.

dead	**MeanDiff**	**Prob**	
TPS-B TPS-C	-0.3828	1	n.s.
S0.01-C TPS-C	9.95626	0.99441	n.s.
S0.01-C TPS-B	10.33906	0.99242	n.s.
S0.01-B TPS-C	-2.8961	1	n.s.
S0.01-B TPS-B	-2.5133	1	n.s.
S0.01-B S0.01-C	-12.8524	0.96215	n.s.
S0.005-C TPS-C	2.91861	1	n.s.
S0.005-C TPS-B	3.3014	1	n.s.
S0.005-C S0.01-C	-7.03766	0.99974	n.s.
S0.005-C S0.01-B	5.8147	0.99996	n.s.
S0.005-B TPS-C	-1.52081	1	n.s.
S0.005-B TPS-B	-1.13801	1	n.s.
S0.005-B S0.01-C	-11.4771	0.98304	n.s.
S0.005-B S0.01-B	1.37528	1	n.s.
S0.005-B S0.005-C	-4.43942	1	n.s.
S0-B TPS-C	25.23779	0.30458	n.s.
S0-B TPS-B	25.62059	0.28567	n.s.
S0-B S0.01-C	15.28153	0.8898	n.s.
S0-B S0.01-B	28.13389	0.18159	n.s.
S0-B S0.005-C	22.31919	0.47248	n.s.
S0-B S0.005-B	26.7586	0.23425	n.s.
S0-C TPS-C	14.99941	0.90079	n.s.
S0-C TPS-B	15.38221	0.88571	n.s.
S0-C S0.01-C	5.04315	0.99999	n.s.
S0-C S0.01-B	17.89551	0.75797	n.s.
S0-C S0.005-C	12.0808	0.97537	n.s.
S0-C S0.005-B	16.52022	0.83365	n.s.
S0-C S0-B	-10.2384	0.99299	n.s.

*TPS, tissue culture polystyrene; S0/0.005/0.01 describe the concentration (mg/ml) of the surfactant pentafluorobenzoyl chloride used in a fluorinated oil (Novec 7500, 0.77 cSt); M, cell culture medium; C, collagen type I; B, BSA. n.s., non-significant; *, P < 0.05; ** P < 0.01; *** P < 0.001.*

Supplementary Table S8. Summary of statistical data obtained for Characterisation of the thickness of protein layers from SEM images (see Supplementary Figure S4D).

	MeanDiff	**Prob**	
s0.005 s0	20.68333	0.46913	n.s.
s0.01 s0	18.53333	0.55492	n.s.
s0.01 s0.005	-2.15	0.99964	n.s.
s0.1 s0	-0.94333	0.99999	n.s.
s0.1 s0.005	-21.6267	0.43426	n.s.
s0.1 s0.01	-19.4767	0.51629	n.s.
s1 s0	9.05	0.92621	n.s.
s1 s0.005	-11.6333	0.84476	n.s.
s1 s0.01	-9.48333	0.91461	n.s.
s1 s0.1	9.99333	0.89982	n.s.

*S0/0.005/0.01/0.1/1 describe the concentration (mg/ml) of the surfactant pentafluorobenzoyl chloride used in a fluorinated oil (Novec 7500, 0.77 cSt). n.s., non-significant; *, P < 0.05; ** P < 0.01; *** P < 0.001.*

Reference

1 Adler, M.; Unger, M; Lee, G. *Pharm. Res.* **2000**, 17, 863-870.