Differentially instructive extracellular protein micro-nets.

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Supporting Information

Abbreviations

All – allyl; DIPEA – diisopropylethylamine; Fmoc – 9-fluorenylmethoxycarbonyl; FTIR –Fourier transform infrared spectroscopy; HBTU – O-benzotriazole N,N,N',N'-tetramethyluronium-hexafluorophosphate; MALDI-ToF – matrix-assisted laser desorption/ionization time of flight; MOPS – 3-(N-morpholino)propanesulfonic acid; QCM-D – quartz crystal microbalance with dissipation; RP-HPLC – reversed phase high pressure liquid chromatography; TIS – triisopropylsilane; TFA – trifluoroacetic acid; XRD – x-ray diffraction.

Tables, Scheme and Figures

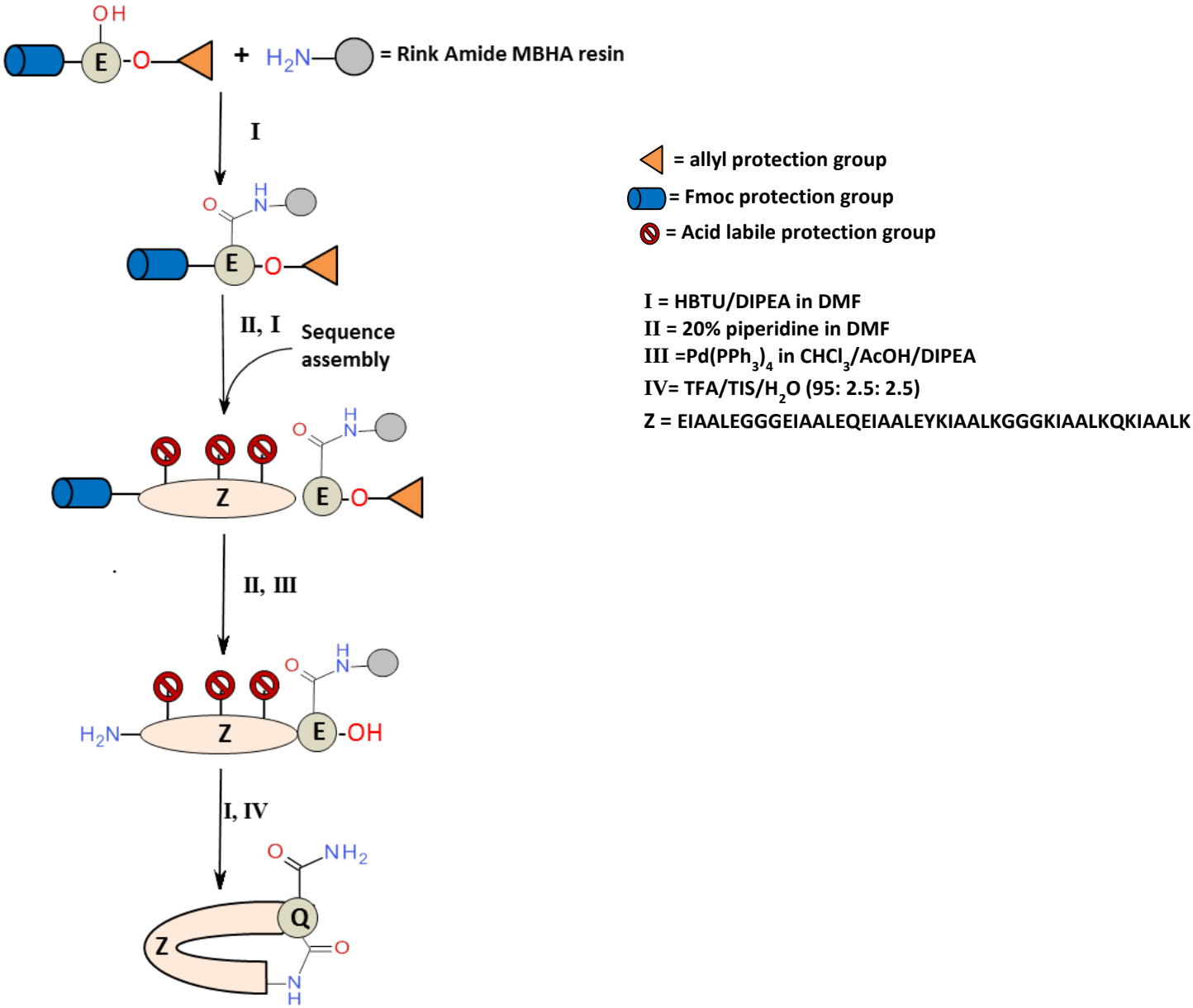
Table S1. Peptide constructs used in the study

Name	Sequence
	gabcdefgabcdefgabcde gabcdefgabcdefgabcde
SaNet	(EIAALEQEIAALEYKIAALK-GGG-KIAALKQKIAALK**Q**EIAALE-GGG)[a]
D1	EIAALEQEIAALEYKIAALK-am
D2	KIAALKQKIAALKYEIAALE-am

[a]*C* - terminal glutamine used for backbone cyclization is highlighted in bold.

Table S2. Biological activity of SaNet

Bacterium	MIC, μM
E. coli (K12)	100
P. aeroginosa (ATCC 27853)	>100
S. aureus (ATCC 6538)	>100
B. subtilis (ATCC 6633)	50
	LC_{50}, μM
Human erythrocytes	>>600



Scheme S1. Synthesis of SaNet cyclopeptide

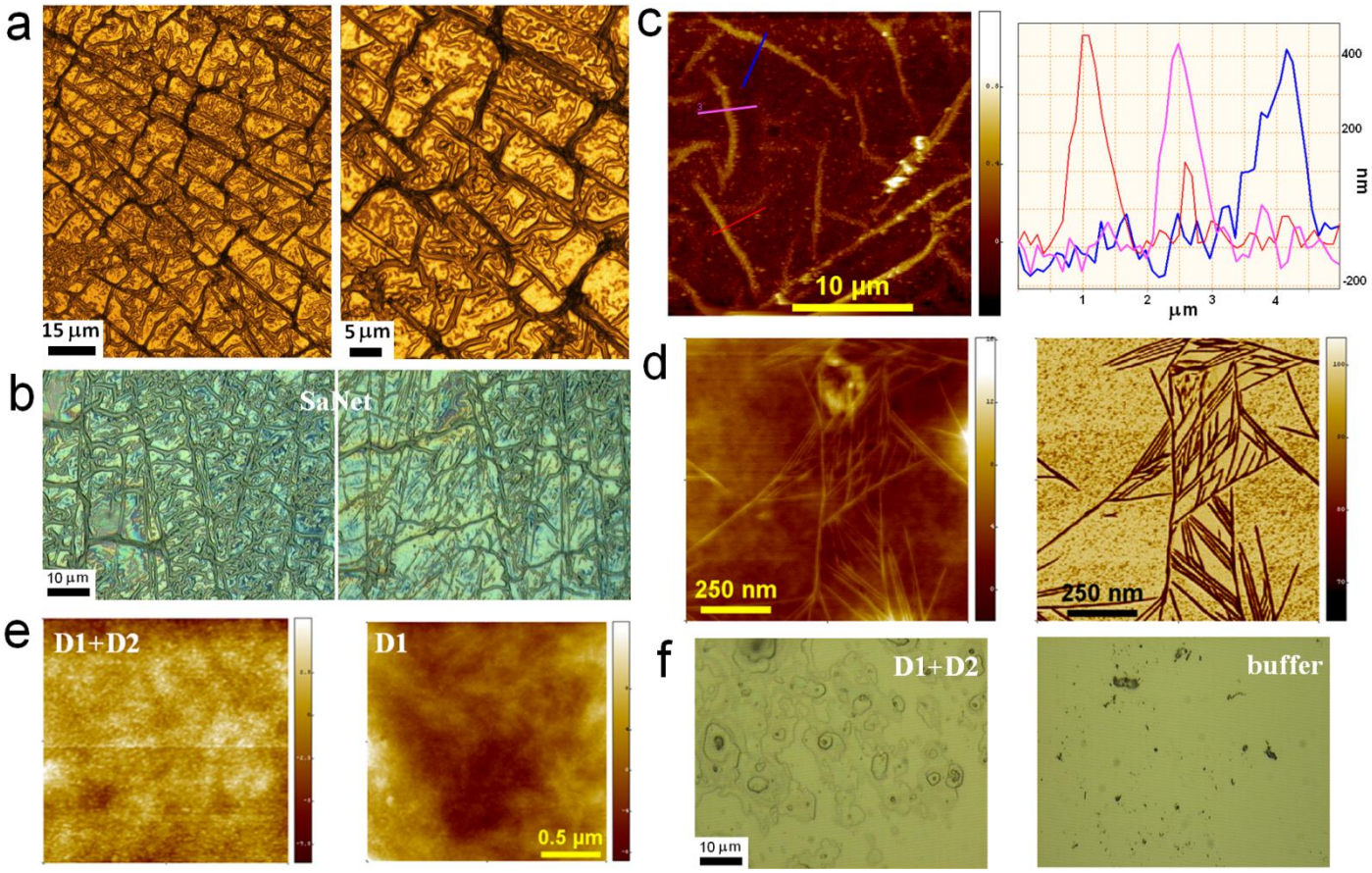


Figure S1. Peptide assembly. Confocal (**a**) and optical (**b**) micrographs of SaNet. AFM images of higher order fibres with cross sections along the highlight lines (**c**) and lower fibrillar branches (**d,** topography image, left, and phase image, right). AFM (**e**) and optical (**f**) micrographs of controls. Assembly conditions: peptide (total 100 µM) incubated overnight at 20°C in 10 mM MOPS buffer, pH 7.4.

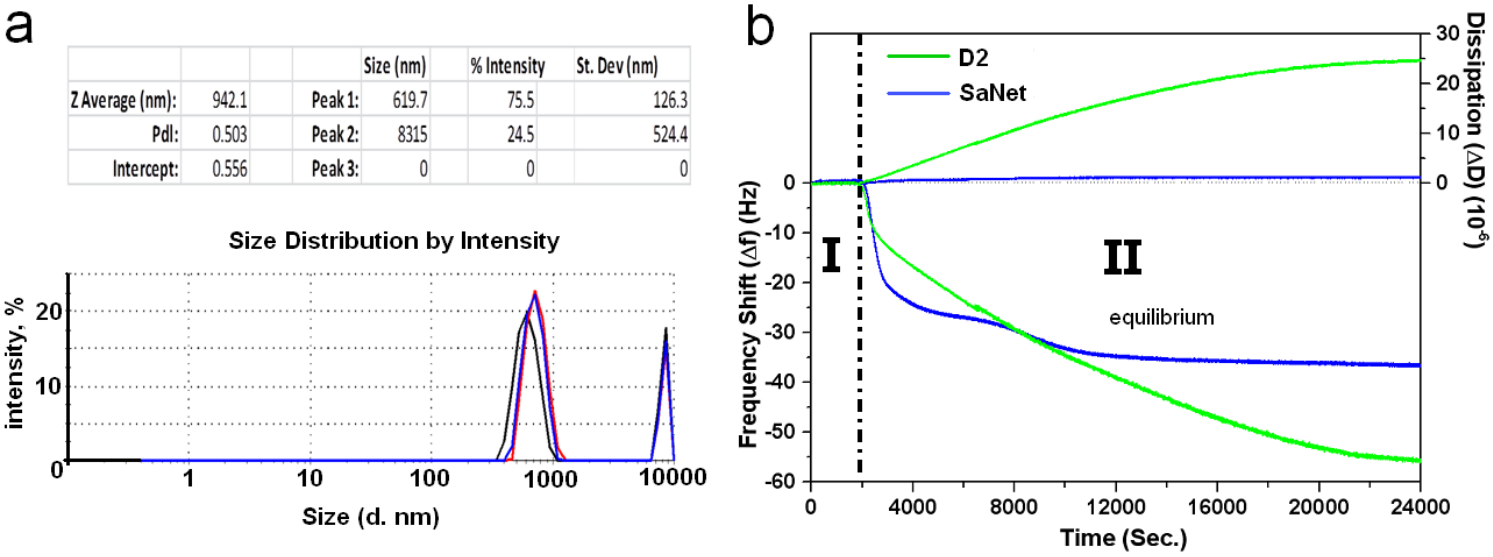


Figure S2. (**a**) Dynamic light scattering traces for SaNet of three independent measurements. The values in the table correspond to the black trace. Assembly conditions: peptide (100 μM) incubated overnight at 20°C in 10 mM MOPS, pH 7.4. (**b**) QCM-D responses (both frequency shift and dissipation) for peptide adsorption on silicon di-oxide coated quartz crystals. Blue and green lines correspond to SaNet and D2, respectively. In the upper half of the figure, changes in dissipation with time due to peptide adsorption are shown and correspond to the right axis. Lower half shows the simultaneous frequency shift of the crystal due to peptide adsorption and relates to the left axis. Regions **I** and **II** correspond to the buffer contribution and peptide adsorption, respectively.

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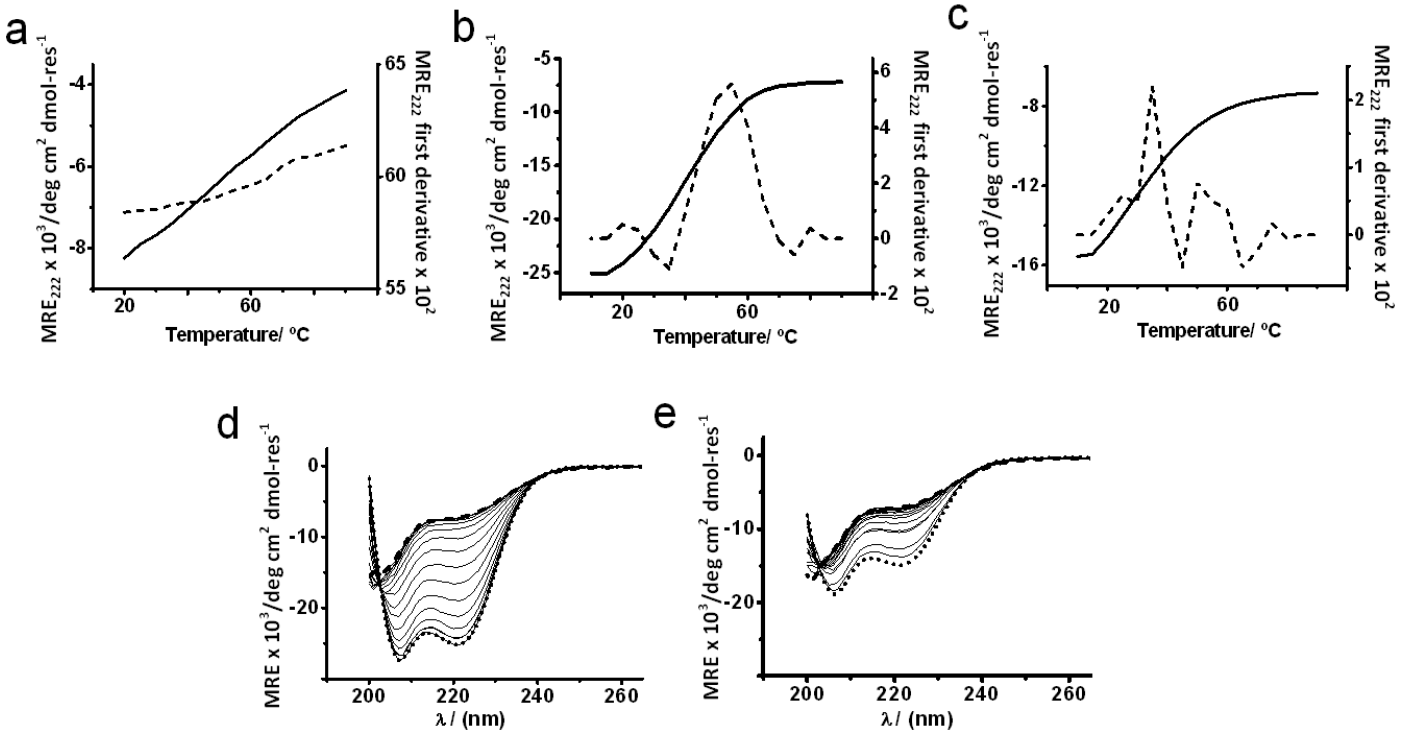

Figure S3. **Thermal unfolding probed by CD spectroscopy.** Thermal denaturation curves (solid lines) and their first derivatives (dashed lines) for SaNet (**a**), D1-D2 (**b**) and D1 (**c**); and CD spectra following the thermal unfolding of D1-D2 (**d**) and D1 (**e**); 10°C (dotted line) and 90°C (dashed line) with intervening spectra recorded every 10°C (solid lines). Note the isodichroic points at ~202 nm. Folding conditions: peptide (total 100 μM) incubated overnight at 20°C in 10 mM MOPS, pH 7.4.

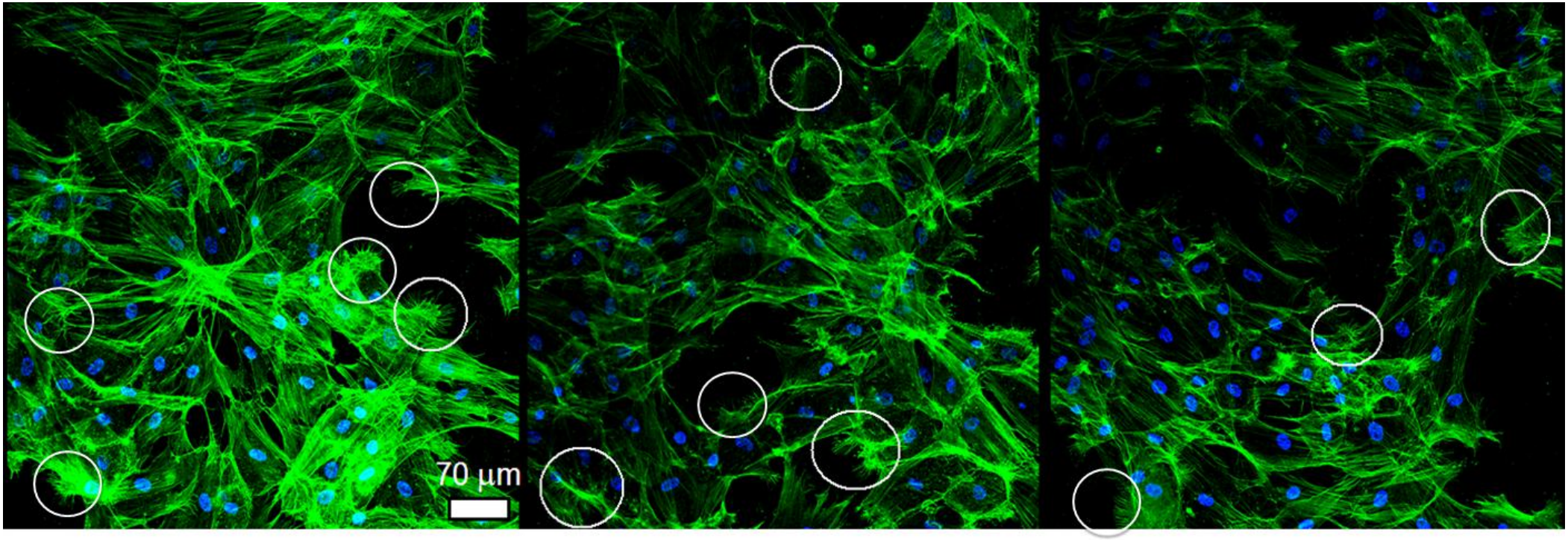

Figure S4. Fluorescence micrographs showing network-like proliferation patterns of human dermal fibroblasts incubated on SaNet for a week. White circles highlight some filopodia protrusions of individual cells.

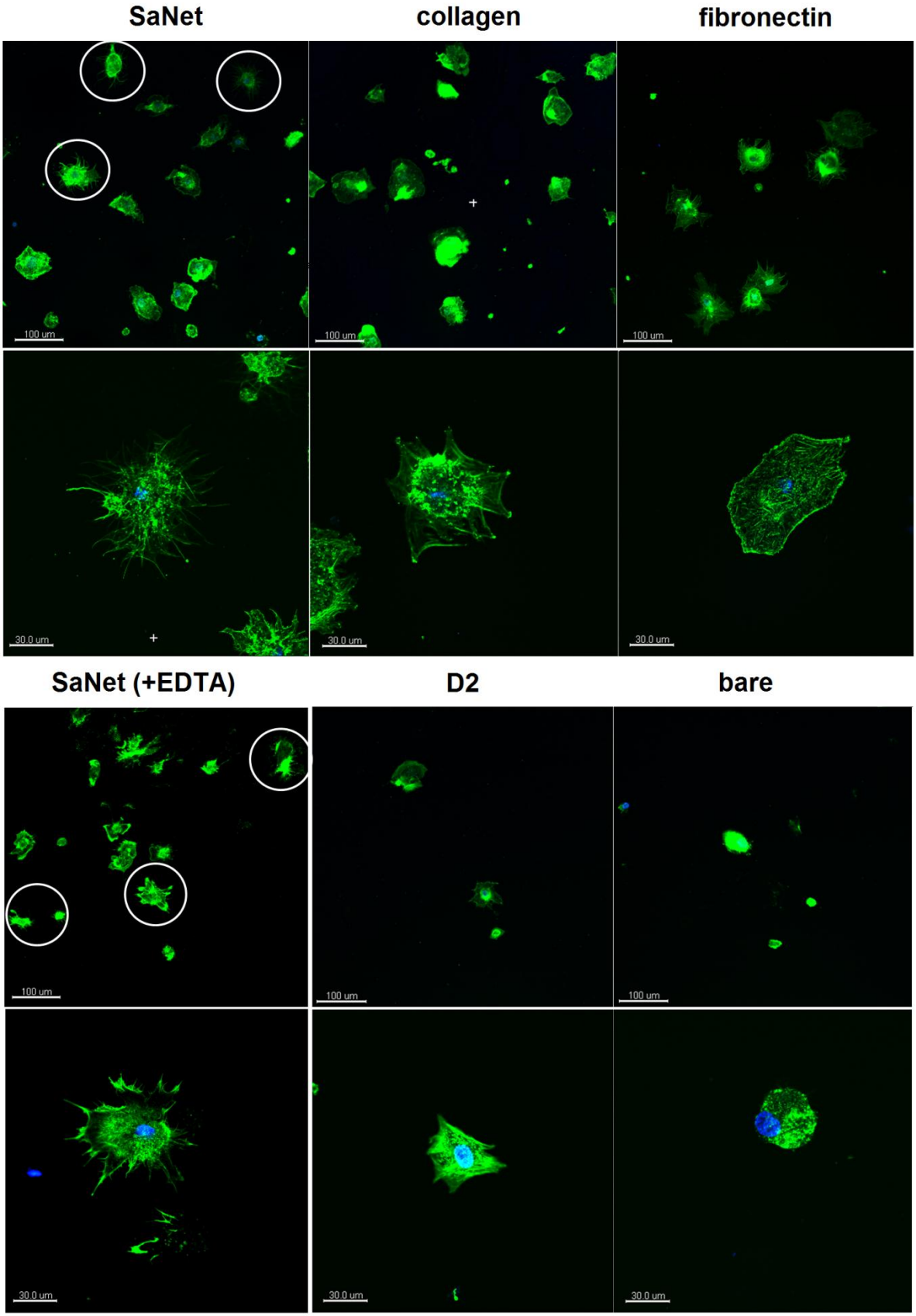

Figure S5. Short-term cell adhesion and spreading. Fluorescence micrographs of human dermal fibroblasts incubated on different substrates for 1 hour. White circles highlight cells with filopodia protrusions.

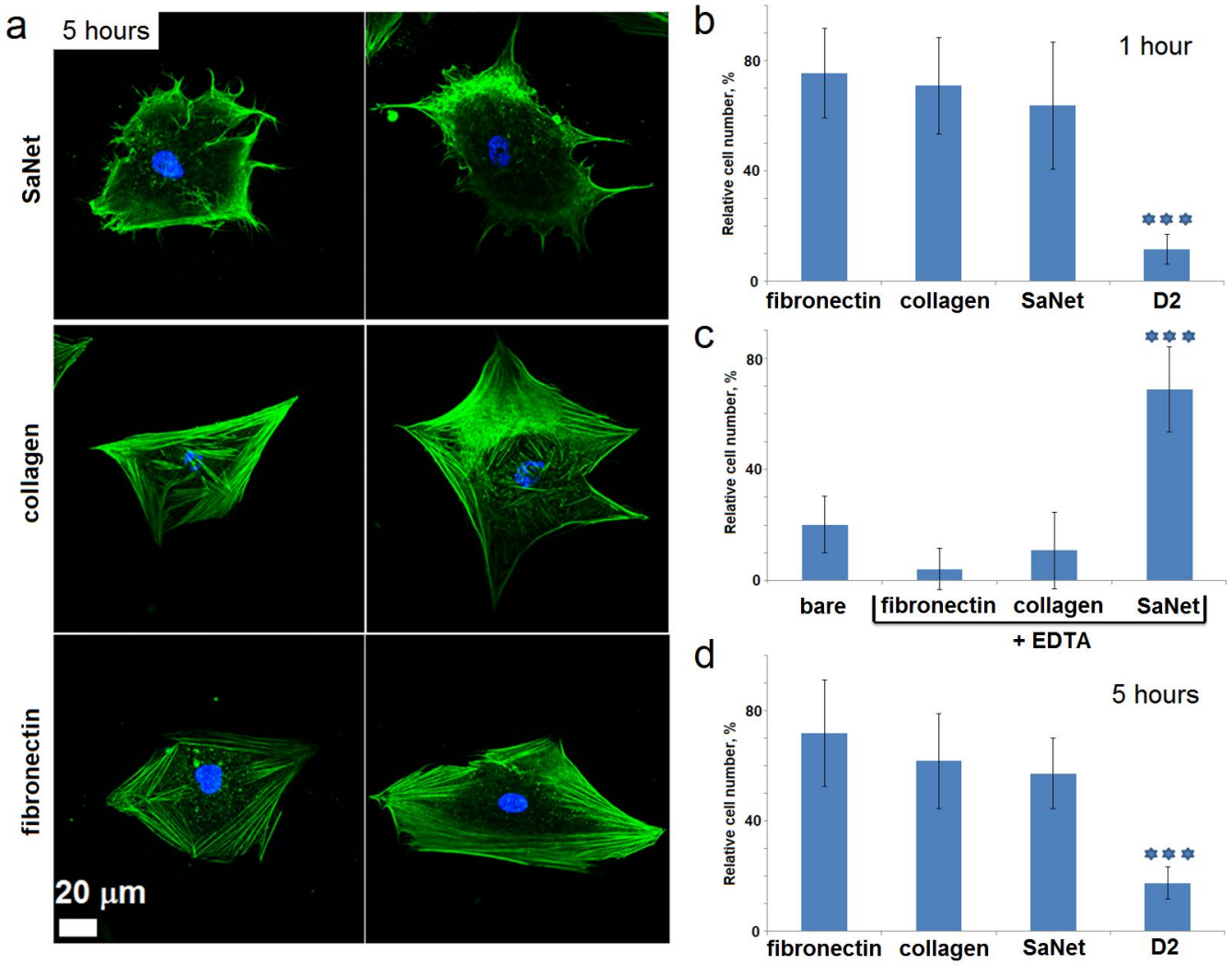

Figure S6. Short-term cell adhesion and spreading. (**a**) Fluorescence micrographs of human dermal fibroblasts incubated on different substrates for 5 hours. (**b**) Relative number of cells attached to each substrate after 1 hour incubation in a serum-free medium after subtracting the background adhesion (bare plastic). D2 had significantly lower numbers of attached cells ($p < 0.0001$) when compared to the other substrates according to ANOVA and multiple means comparison tests. (**c**) Relative number of cells attached to fibronectin, collagen and SaNet substrates after 1 hour incubation in a serum-free medium with the addition of EDTA (10 mM). Background adhesion to bare substrates is without EDTA. SaNet with EDTA had significantly higher numbers of attached cells ($p < 0.0001$) when compared to all the other substrates according to ANOVA and multiple means comparison tests. (**d**) Relative areas of cells spread on each substrate after a 5-hour incubation in a serum-free medium. D2 had significantly less cell spread areas ($p < 0.0001$) when compared to the other substrates according to ANOVA and multiple means comparison tests.

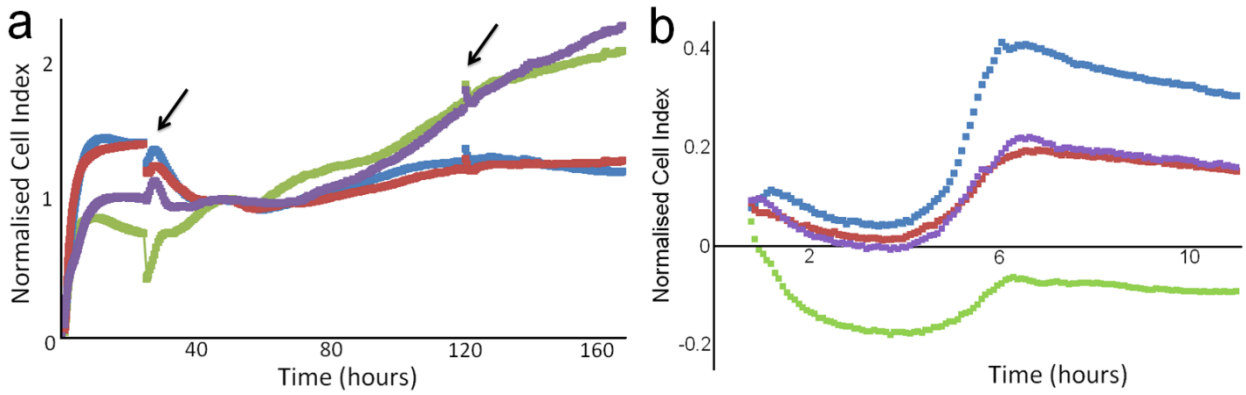

Figure S7. Normalized impedance of HDFs (a) and *B. subtilis* (b) grown on collagen (purple), SaNet (green), fibronectin (red) and bare (blue) substrates. Arrows indicate second and third media inoculations.

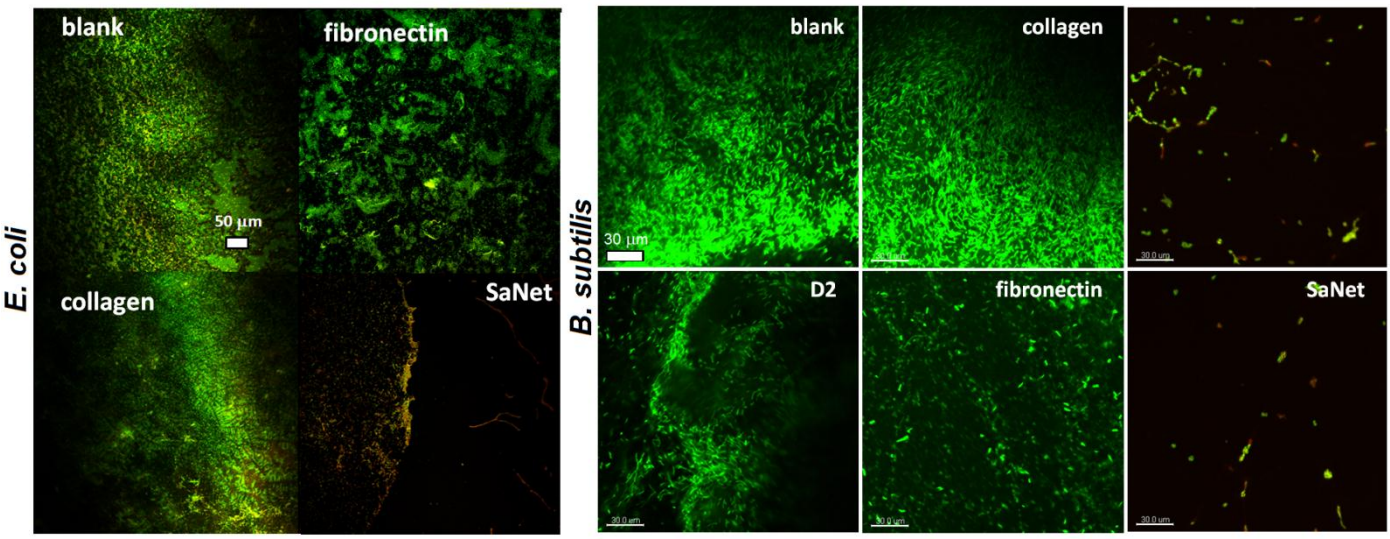

Figure S8. *E. coli* and *B. subtilis* colonization. Fluorescence micrographs of bacterial cells seeded on different substrates – live/dead staining was used to label live (green) and dead (red) bacteria.

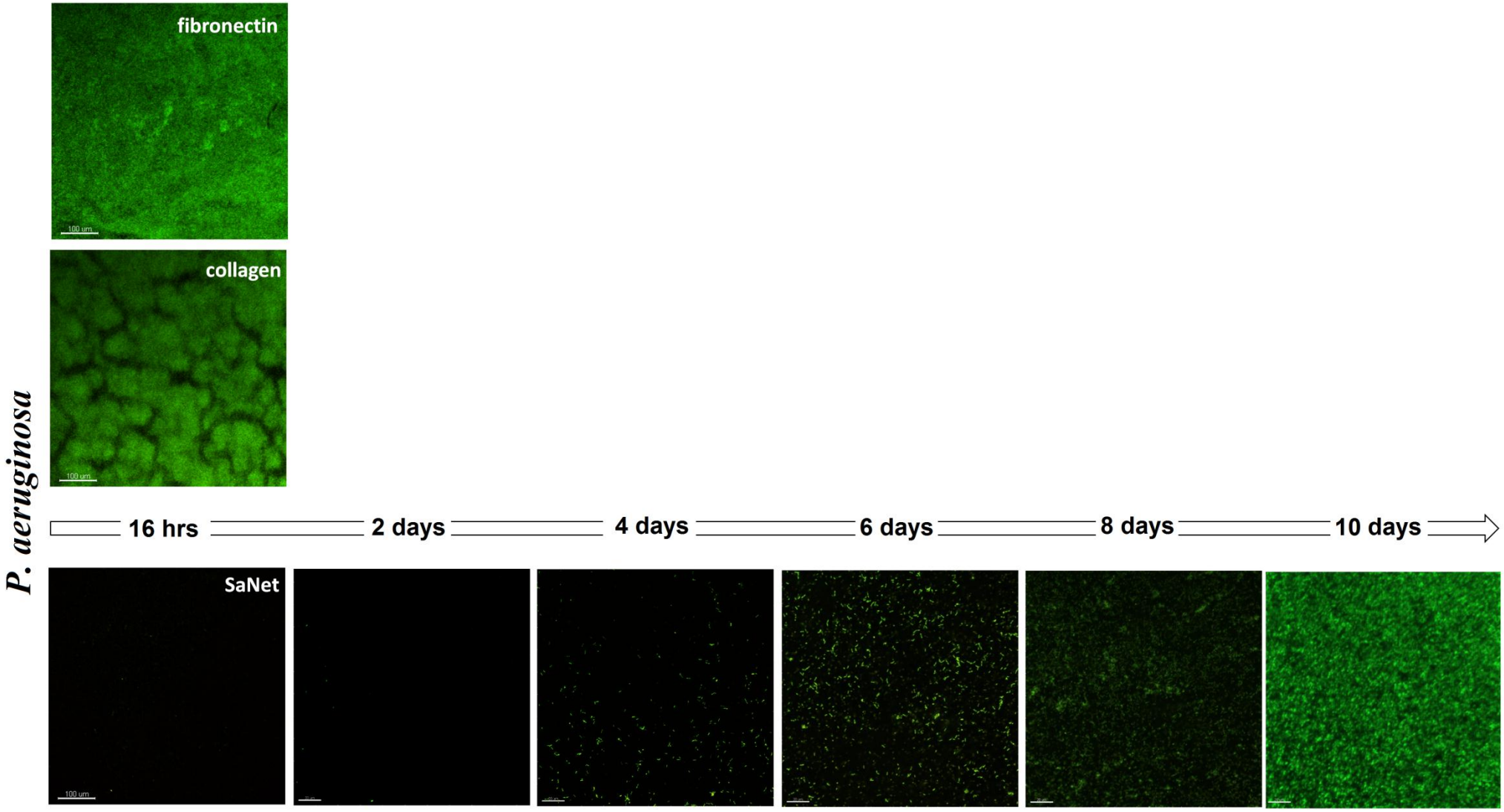

Figure S9. *P. aeruginosa* colonization. Fluorescence micrographs of bacterial cells incubated on SaNet substrates over 10 days – live/dead staining was used to label live (green) and dead (red) bacteria. Micrographs were taken at different time points. The micrographs for bacteria incubated over 16 hours on collagen and fibronectin substrates used as controls are given for comparison.