Supporting Information

The Janus role of adhesion in chondrogenesis

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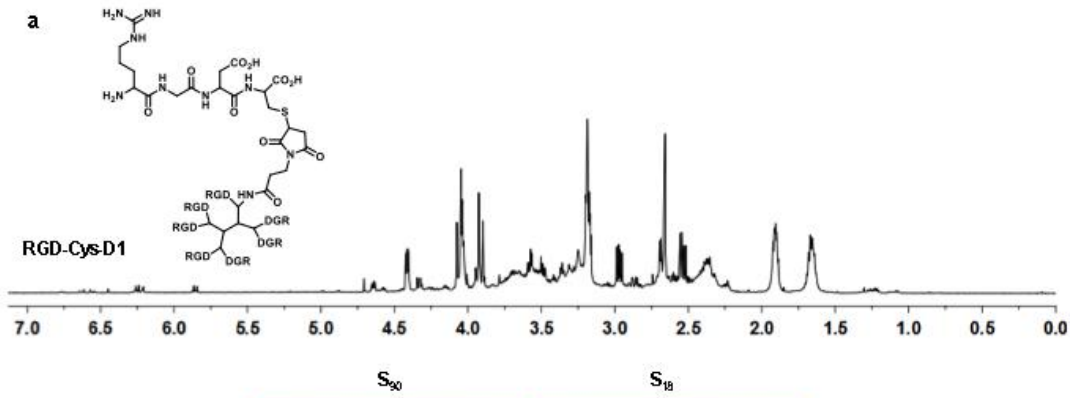
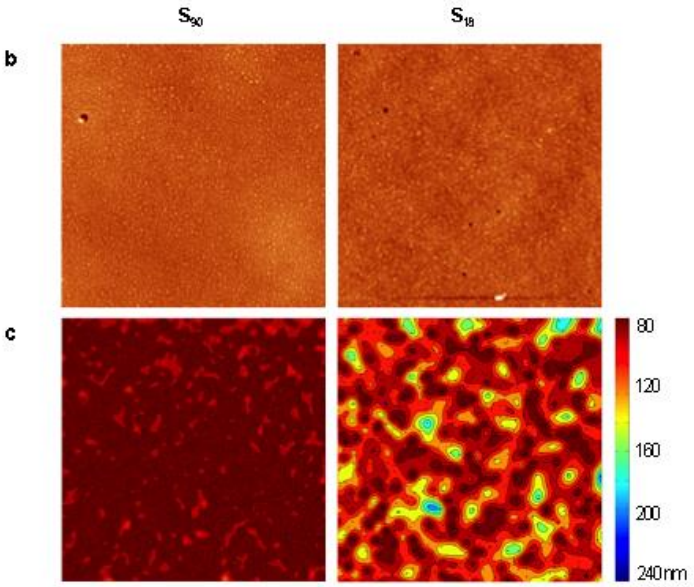
Supplementary Materials and Methods

Cell viability assay

Cells were cultured on nanopatterned or fibronectin-coated substrates as described in the Materials and Methods section. A commercial viability/cytotoxicity cell labelling kit was used for the assay (ThermoFisher, L3224). At day 7 of chondrogenesis, samples were washed twice with PBS and stained with calcein AM and ethidium homodimer-1 at 4 μM in non-serum containing medium for 40 min at 37°C and 5% CO_2. Samples were washed twice with PBS, mounted on microscopy slides, and immediately imaged in a Leica SPE Upright Confocal Microscope (Leica Microsystems) with a 40X objective. Z-projections of cell condensates were produced with Fiji software.





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Nanopattern	RGD-Cys-D1 (% w/w)	Area of d_{min} < 70nm (%)
S_{90}	2510^{9}	90±2
S_{18}	410^{9}	18±11

Figure S1. Nanopatterns of high and low local surface adhesiveness. (a) ^{1}H-NMR spectra of RGD-Cys-D1, in D_2O. Signals between 1.5 and 2.0 ppm were attributed to internal signals of the arginine (R) moiety. The remaining RGD-Cys signals overlapped those corresponding to the dendrimer (between 2.0 and 5.0 ppm). The absence of signals around 7 ppm indicates that all maleimide groups were functionalized. The spectra were acquired on a Bruker Avance 600 MHz spectrometer equipped with a 5 mm TXI inverse probe. (b) Representative atomic force microscopy (AFM) height images of the nanopatterns of RGD-functionalized dendrimers on PLLA (5x5 μm). (c) Minimum interparticle distance (d_{min}) probability contour plots, showing high-density RGD regions in dark red (d_{min} < 70 nm). Dendrimers localization is superimposed in black for clarity. (d) Percentage of area with d_{min} < 70 nm obtained for nanopatterns of high (S_{90}) and low (S_{18}) local surface adhesiveness.

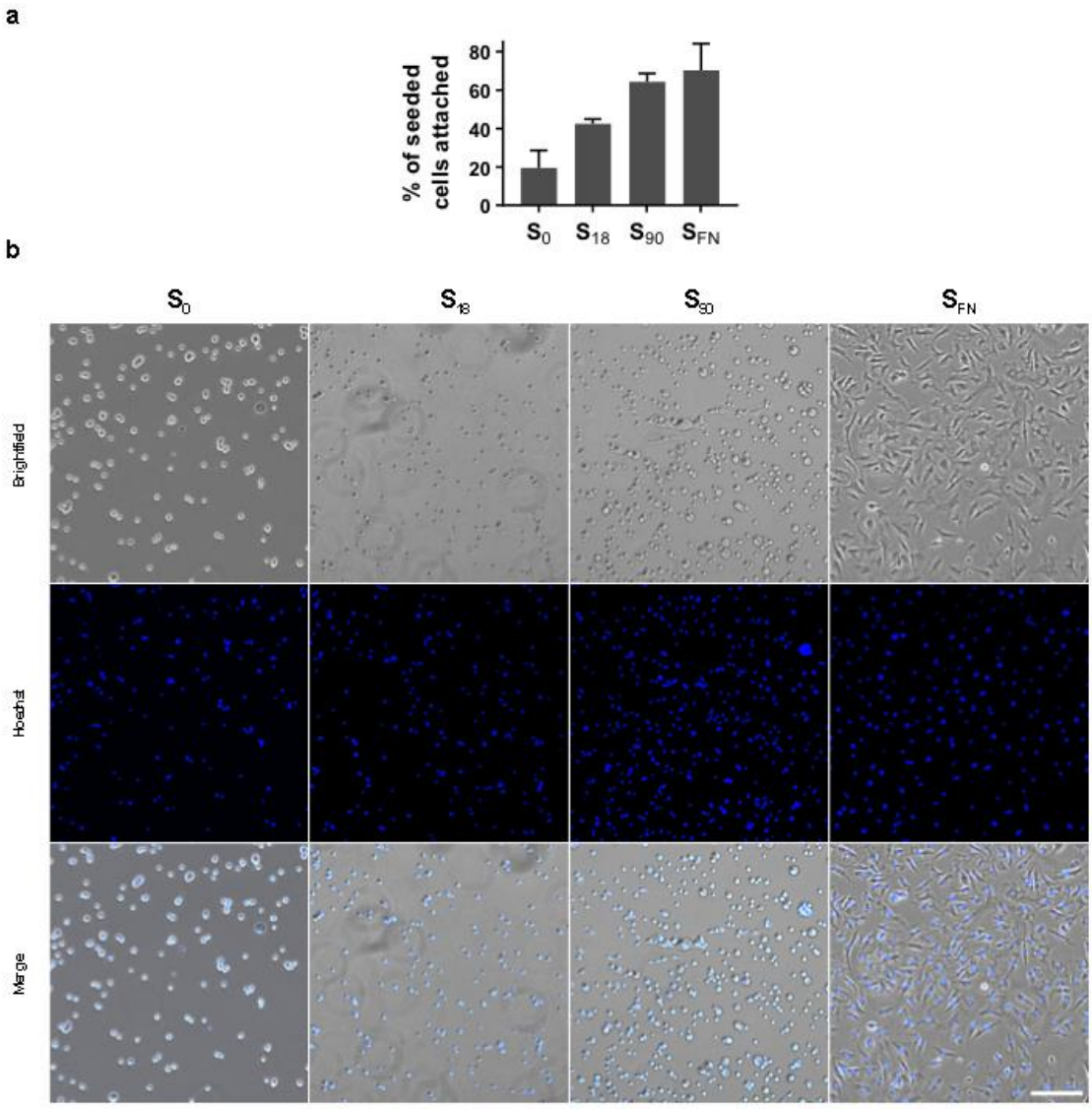

Figure S2. Initial cell adherence on the substrates. (a) Plot of the percentage of adhered cells 2 h after cell seeding. N = 2. Results are given as the mean ± SE. (b) Representative images of the adhered cells. Scale bar = 200 μm**.**

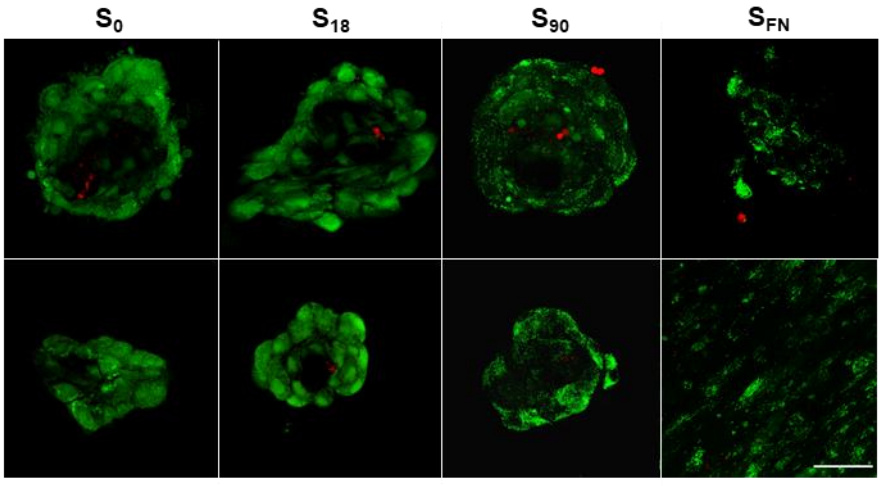

Figure S3. Cell viability assay. Samples at day 7 of chondrogenesis were stained for intracellular esterase activity, indicating live cells (green), and for nucleic acids in membrane-damaged cells (red). Two representative images are shown for each condition. Scale bar = 50 μm.

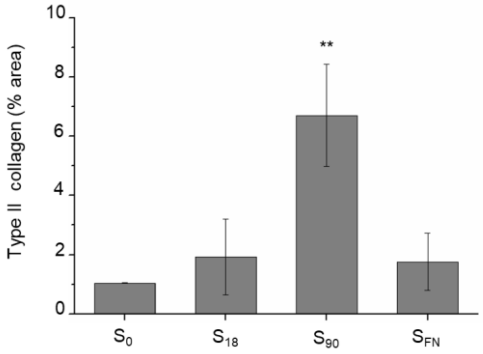

Figure S4. Type II collagen quantification. Plot of the percentage of COL2A1-stained areas in the confocal z-projections, normalized to the area of the condensate after 5 days of chondrogenic induction. N ≥ 3. Results are given as the mean ± SE. The Type II collagen expression in S_{90} nanopatterns is significantly higher than in the rest of the samples.

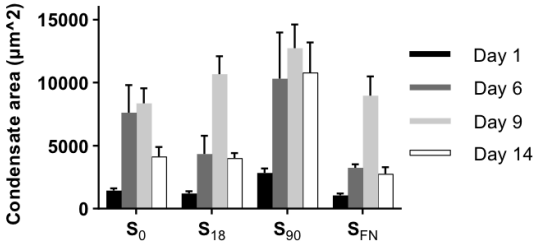

Figure S5. Condensate areas obtained from confocal image projections at different time points of chondrogenesis. N ≥ 3. Results are given as the mean ± SE. Condensates on S_{90} grow larger and are more stable in time than those on the other substrates.