Supporting Information

The Effect of Size and Geometry of Poly(acrylamide) Brush-Based Micro-Patterns on the Behavior of Cells

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Experimental Section[1,2]

Materials and Methods

Materials

Microscopy glass slides were purchased from Thermo Scientific, Menzel-Gläser (ISO 8037/1; 76 mm × 26 mm). Gold was purchased from Allgemeine Gold- und Silberscheideanstalt AG (Pforzheim); 99.99% (granules), Titanium from Chempur; 99.998% (pieces: 1-6mm), ethanol from Fischer Scientific, 97%, denaturated, methanol from J.T. Baker. Cu(I)Br was synthesized according to the literature.[3] 1,1,4,7,7, PMDETA [pentamethyldiethylene triamine] and AAm [acrylamide] (99.9%) was purchased from Sigma Aldrich and was used as received for the PAAm brush synthesis. The initiator, MUBiB [ω-mercaptoundecyl bromoisobutyrate], was synthesized according to Jones et al.[4] ODT [1-octadecanethiol] was purchased from Sigma Aldrich (99%). Milli-Q water was drawn from a Millipore Direct Q8 system (Millipore Advantage A 10 system, Schwalbach, with a Millimark Express 40 filter, Merck, Germany) with a resistivity of 18.0 MΩcm.

Gold Substrate Preparation[1]

Gold substrates were prepared by electron beam evaporation under high vacuum (Edwards E306 coating system, Moorfield, UK).[5] Before any further use, they were cleaned using a BioForce UV/ozone Pro Cleaner (BioForce Nanosciences, Ames, USA) for 30 min.

Culture of NIH 3T3 fibroblasts and Patu 8988T cells[1]

NIH 3T3 mouse fibroblasts and Patu 8988T cells were cultured at standard conditions (37°C, 5% CO_2) in Dulbecco's modified Eagle's medium (DMEM; Gibco, Life Technologies, Carlsbad, USA) supplemented with 10% fetal bovine serum (Gibco, Life Technologies), 1% L-Glutamine (2 mM; Gibco, Life Technologies), 1% penicillin (100 /mL; Gibco, Life Technologies) and streptomycin (100 µg/mL; Gibco, Life Technologies). For PatuT cells the medium was supplemented with 5% fetal bovine serum and 5% horse serum (Gibco, Life

Technologies). For passaging, the cells were rinsed with PBS, detached by using 0.25 % trypsin/EDTA (Gibco, Life Technologies), resuspended and diluted in fresh DMEM. Then the cell pellets were collected by centrifugation (1200 rpm for 4 min) in a conical tube. The final concentration of the cells was calculated with a Neubauer improved counting chamber (Brand, Wertheim, Germany).

Cell Seeding[1]

Cells, seeded at a density of 15,000 cells/ cm^2 in 3 mL medium per well, were incubated at 37°C.[1] Further analysis by optical microscopy (Primovert, Carl Zeiss, Oberkochen, Germany) after 6 h adhesion time followed upon thoroughly rinsing the samples with fresh medium. In the case of long time cell adhesion assays, the substrates were incubated with cells for two days, rinsed with PBS and again covered with fresh medium. This procedure was repeated every second day, during the desired period of time.

Surface Characterization[1]

X-ray photoelectron spectroscopy (XPS) was carried out on a ESCA spectrometer (S-probe ESCA SSX-100S Surface Science Instruments, USA) with Al Kα X-ray radiation of 200 W.

Fourier transform infrared (FTIR) spectra were obtained on a IFS 66v spectrometer (Bruker, Billerica, USA) with a VEEMAX-II grazing angle accessory (Pike Technologies, Fitchburg, USA).

Ellipsometry measurements were performed with an alpha-SE ellipsometer (J. A. Woollam Co., Inc., Lincoln, USA) at three different incidence angles (65°, 70° and 75°) with wavelengths between 380 and 900 nm.

Time of flight-secondary ion mass spectroscopy (ToF-SIMS) data were acquired with an IONTOF.4 (Münster, Germany) using a 25 keV Bi^+ primary ion source.

Static and dynamic water contact angles were measured with a 2 µL drop of Milli-Q water at ambient conditions using an OCA-15 instrument (Dataphysics, Filderstadt, Germany).

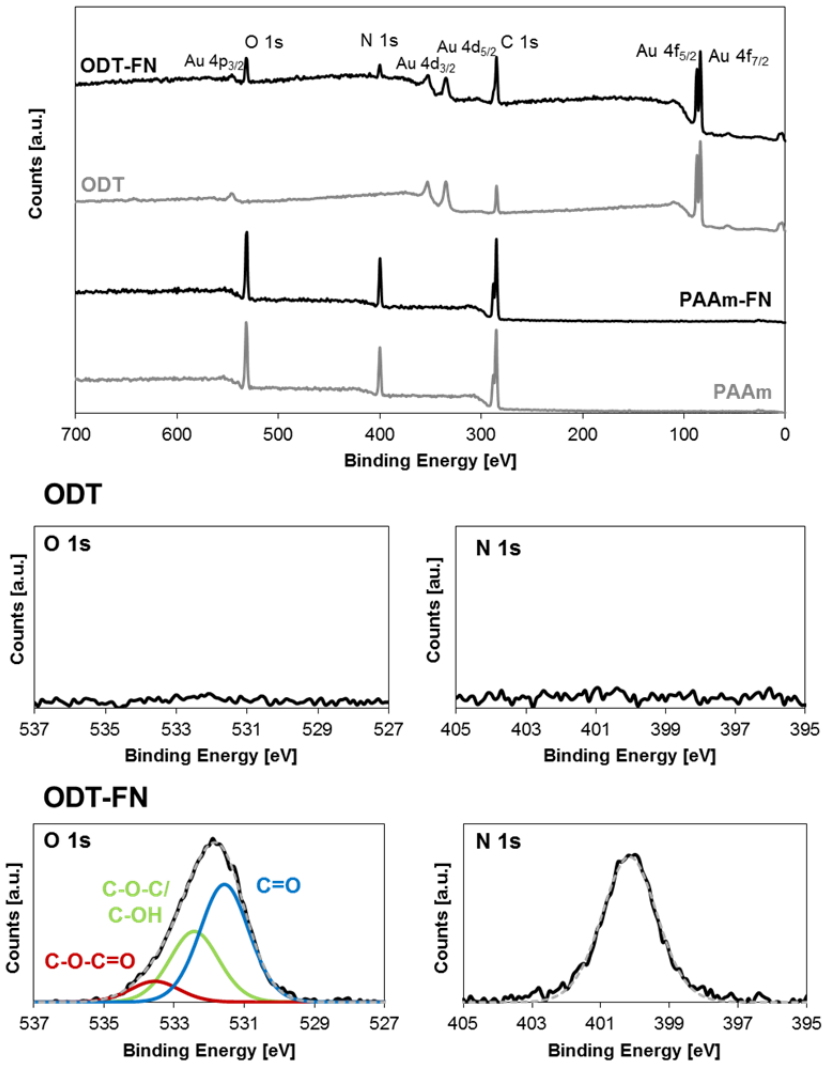


Figure S1: XPS survey spectra of PAAm brushes (dry ellipsometric thickness: 45nm) and ODT before and after FN adsorption.

Table S1: Atomic concentrations of the different components of PAAm brushes (dry ellipsometric thickness: 45 nm) and ODT before and after FN adsorption obtained from the C 1s and O 1s high resolution peak.

	C 1s [atom-%]				O 1s [atom-%]		
Sample	C-C	C-N/C-S	C-O	N-C=O	C=O	C-O-C/C-O-H	C-O-C=O
	285.0 eV	286.0 eV	286.8 eV	288.3 eV	532.2eV	532.8 eV	533.6 eV
PAAm	70.9	-	-	29.1	100.0	-	-
PAAm-FN	71.4	-	-	28.6	100.0	-	-
ODT	94.3	5.7	-	-	-	-	-
ODT-FN	61.2	18.8	8.8	11.2	33.8	9.99	56.21

Table S2: Dry ellipsometric thickness and static contact angles of PAAm brushes and ODT before and after exposure to a FN solution for 60min.

Sample	Thickness [nm]	Static Contact Angle [degrees]
ODT	2.0 ± 0.2	110 ± 1
ODT-FN	5.0 ± 0.3	69 ± 3
PAAm	42 ± 1.3	22 ± 1
PAAm-FN	41 ± 1.7	23 ± 2

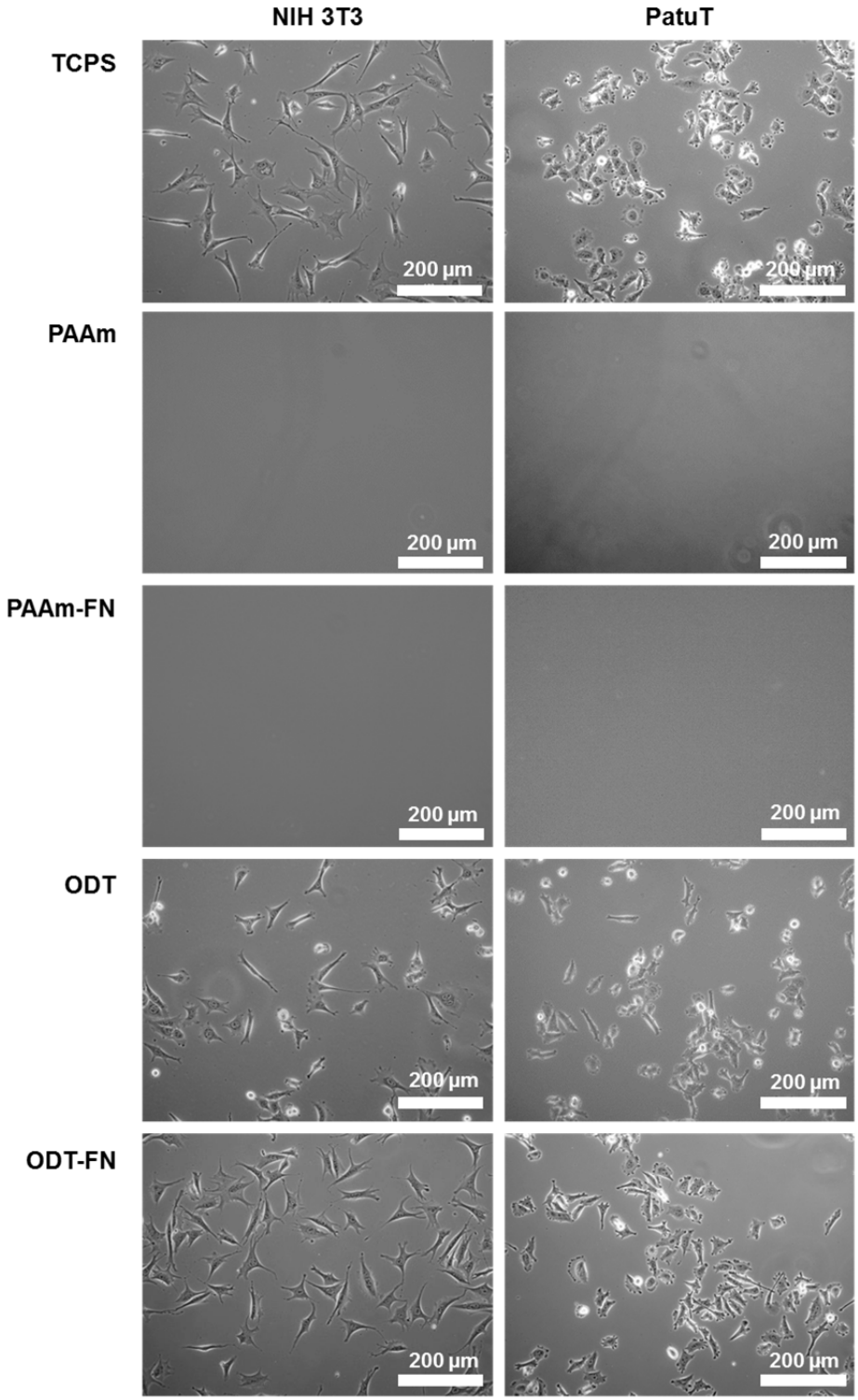


Figure S2: Optical microscopy images of NIH 3T3 and PatuT cells on PAAm brushes and ODT SAMs with and without FN pretreatment.

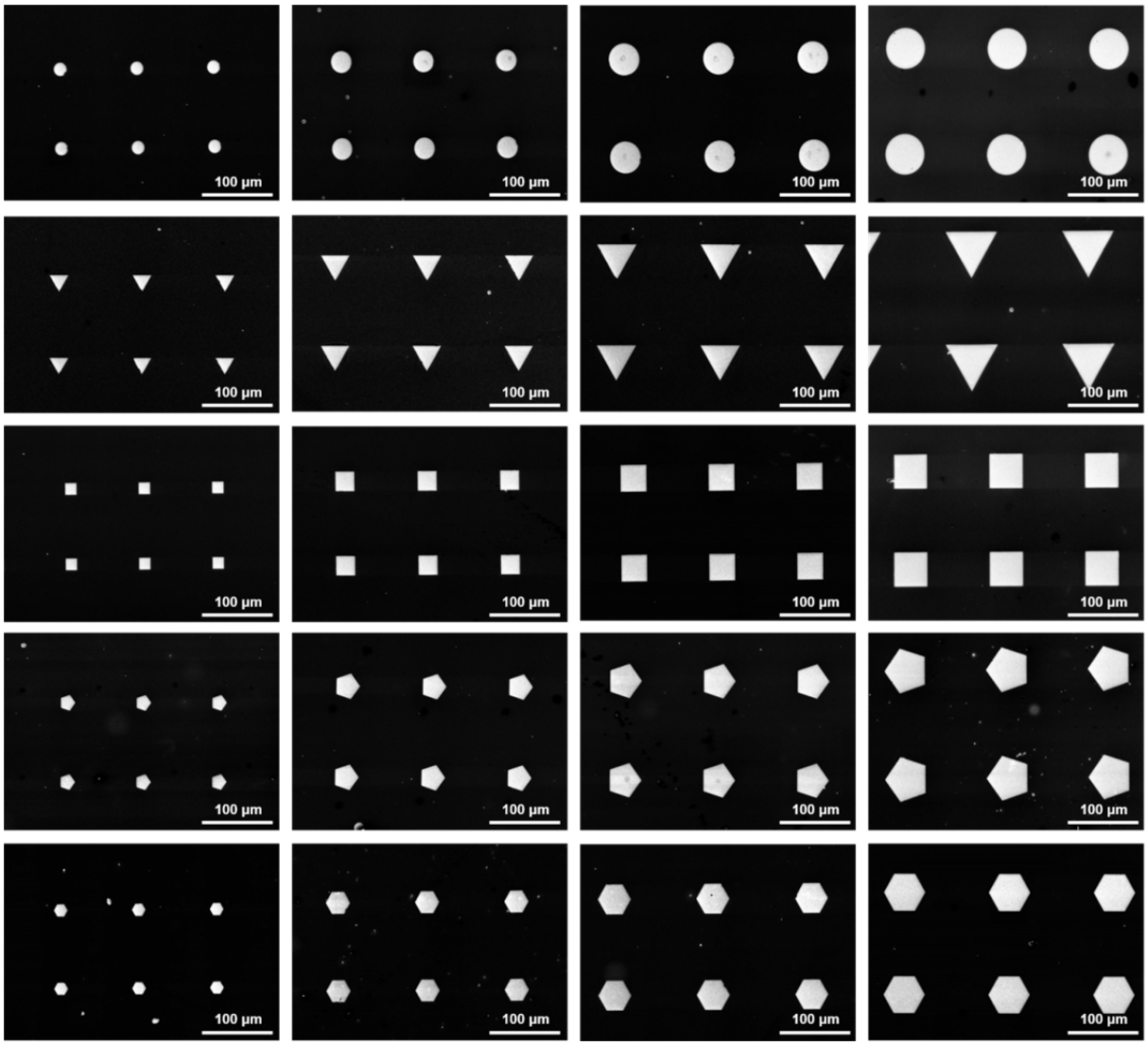


Figure S3: SEM images of micro-patterned PAAm brushes on gold substrate with increasing adhesive area from left to right (400, 900, 1600 and 2500 µm^2).

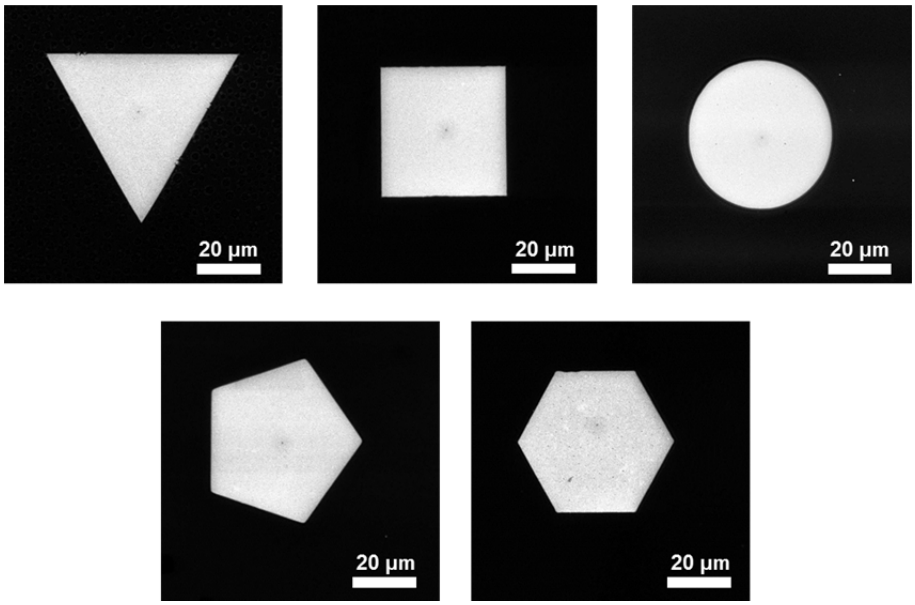


Figure S4: SEM images of triangular, square, circular, pentagonal and hexagonal PAAm brush micro-patterns with an adhesive area of 1600 μm^2.

Table S3: Average adhesive areas of the different PAAm brush micro-patterns measured by SEM and analyzed by imageJ.

	Circle		Triangle		Square		Pentagon		Hexagon	
Area [µm²]	**Area [µm²]**	**%**	**Area [µm²]**	**%**	**Area [µm²]**	**%**	**Area [µm²]**	**%**	**Area [µm²]**	**%**
400	262 ± 29	66	340 ± 31	85	295 ± 25	74	323 ± 36	81	278 ± 12	69
900	809 ± 54	90	819 ± 31	91	804 ± 29	89	789 ± 13	88	857 ± 46	95
1225	1155 ± 98	92	1099 ± 77	90	1099 ± 99	90	1203 ± 72	98	1010 ± 84	82
1600	1485 ± 175	93	1578 ± 57	99	1534 ± 85	96	1457 ± 87	91	1572 ± 123	98
2025	1959 ± 178	97	1901 ± 123	94	1899 ± 117	94	2008 ± 139	99	1954 ± 142	96
2500	2467 ± 214	99	2361 ± 173	94	2419 ± 159	97	2498 ± 194	100	2395 ± 184	96

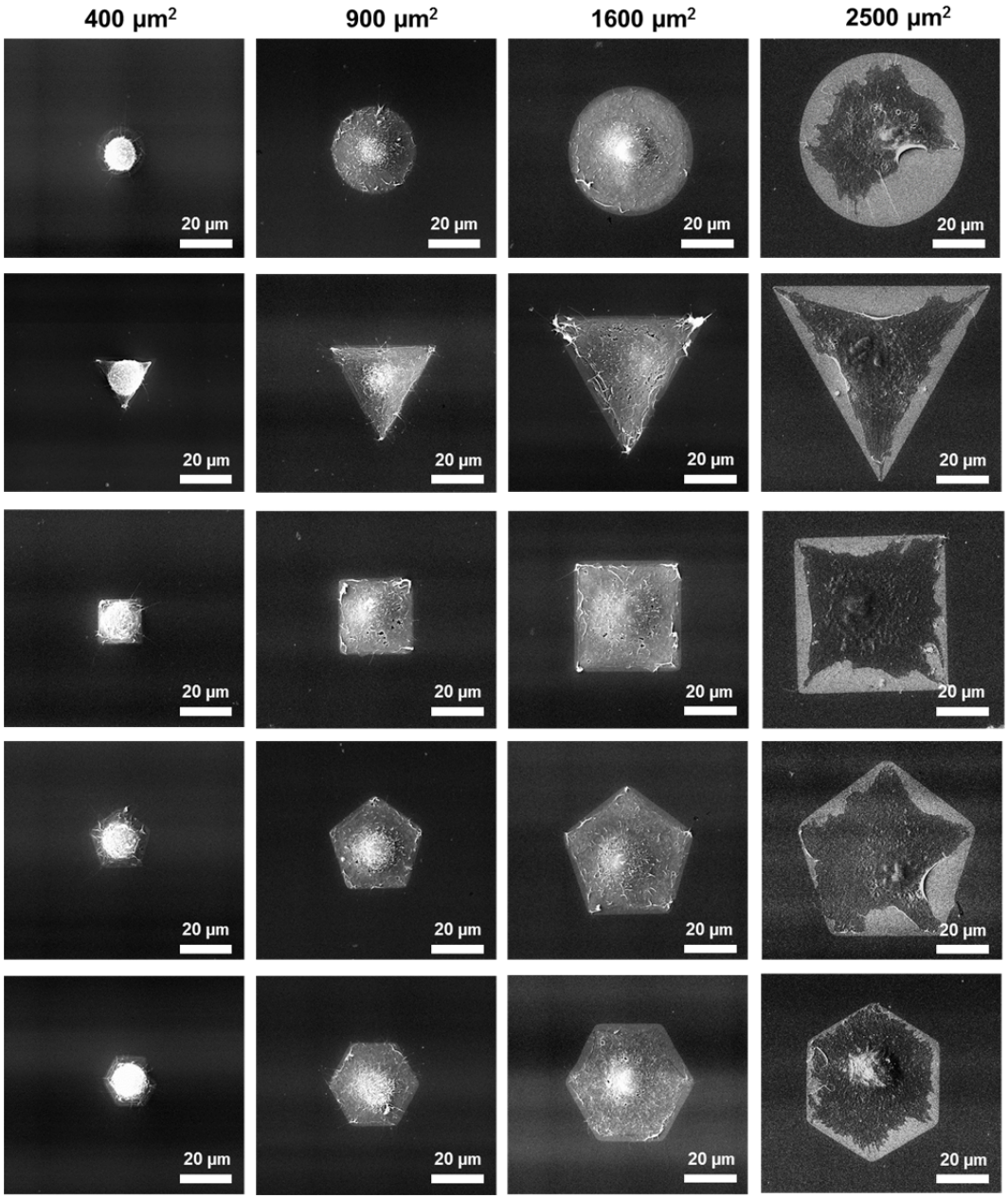


Figure S5: SEM images of NIH 3T3 Cells on FN-coated patterns with varied adhesive areas (increasing from left to right; 400, 900, 1600 and 2500 μm^2) and with different geometries.

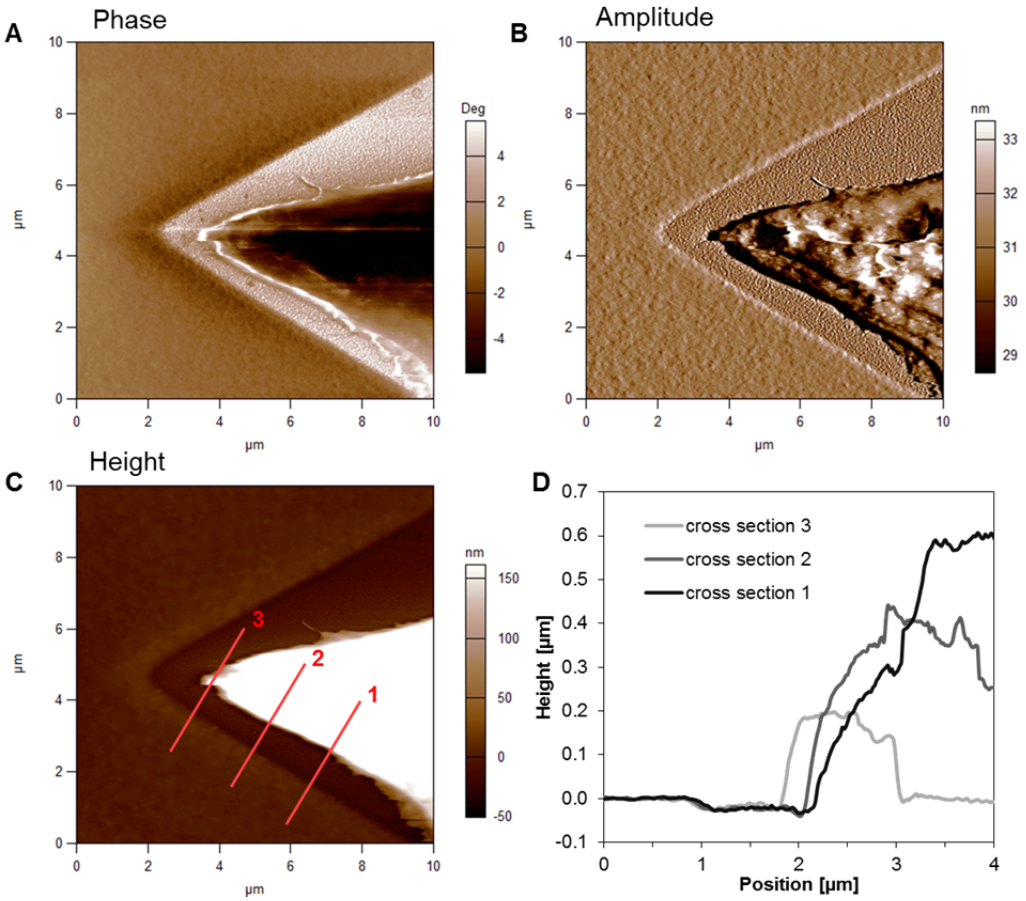


Figure S6: AFM analysis of a representative NIH 3T3 cell on a triangular PAAm brush pattern fixed with glutaraldehyde (3 wt%) in PBS. A: phase image. B: amplitude image. C: height image with respective cross sections, which are displayed in D.

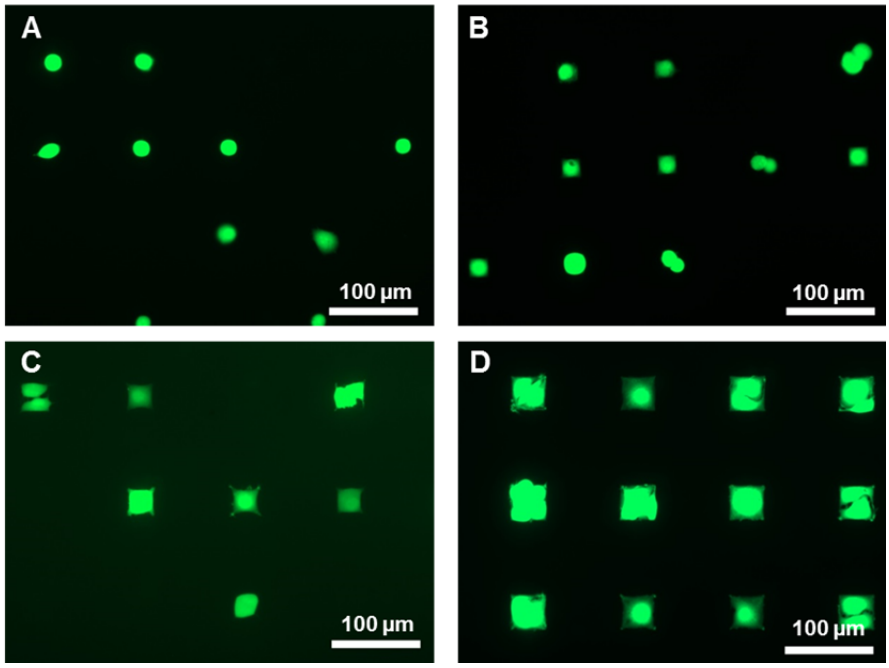


Figure S7: Cell viability test (FDA and PI), 6h after cell seeding (NIH 3T3 cells; A: 400 μm^2; B: 900 μm^2; C: 1600 μm^2 and D: 2500 μm^2).

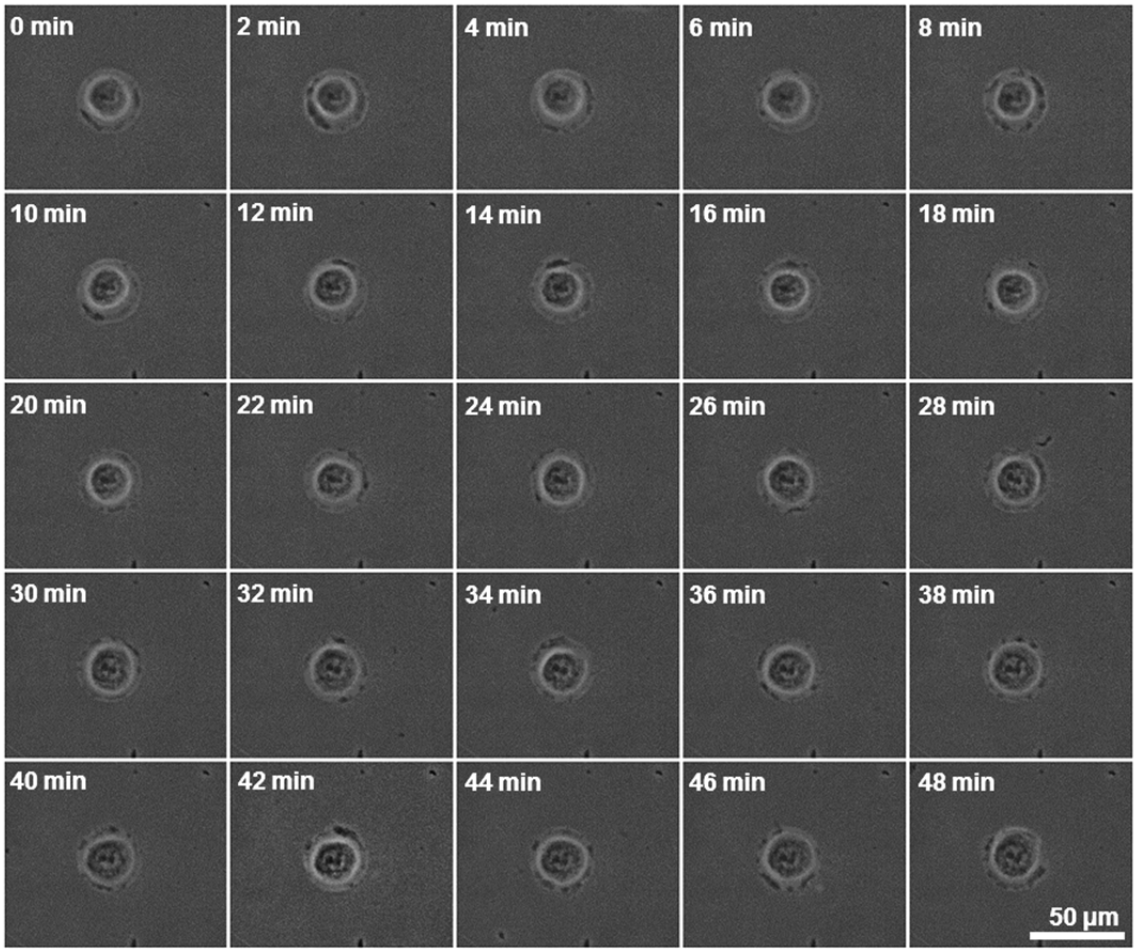
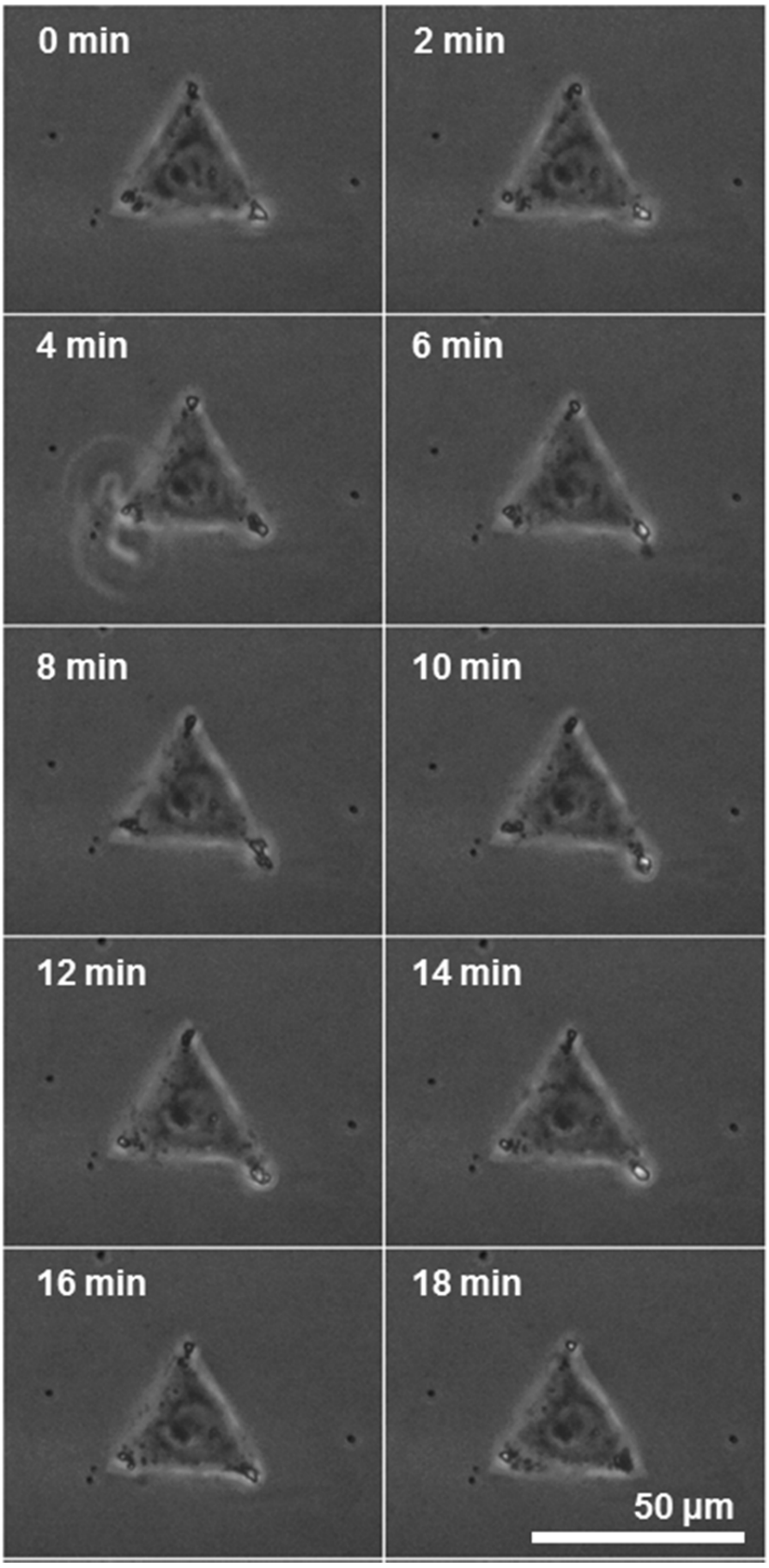
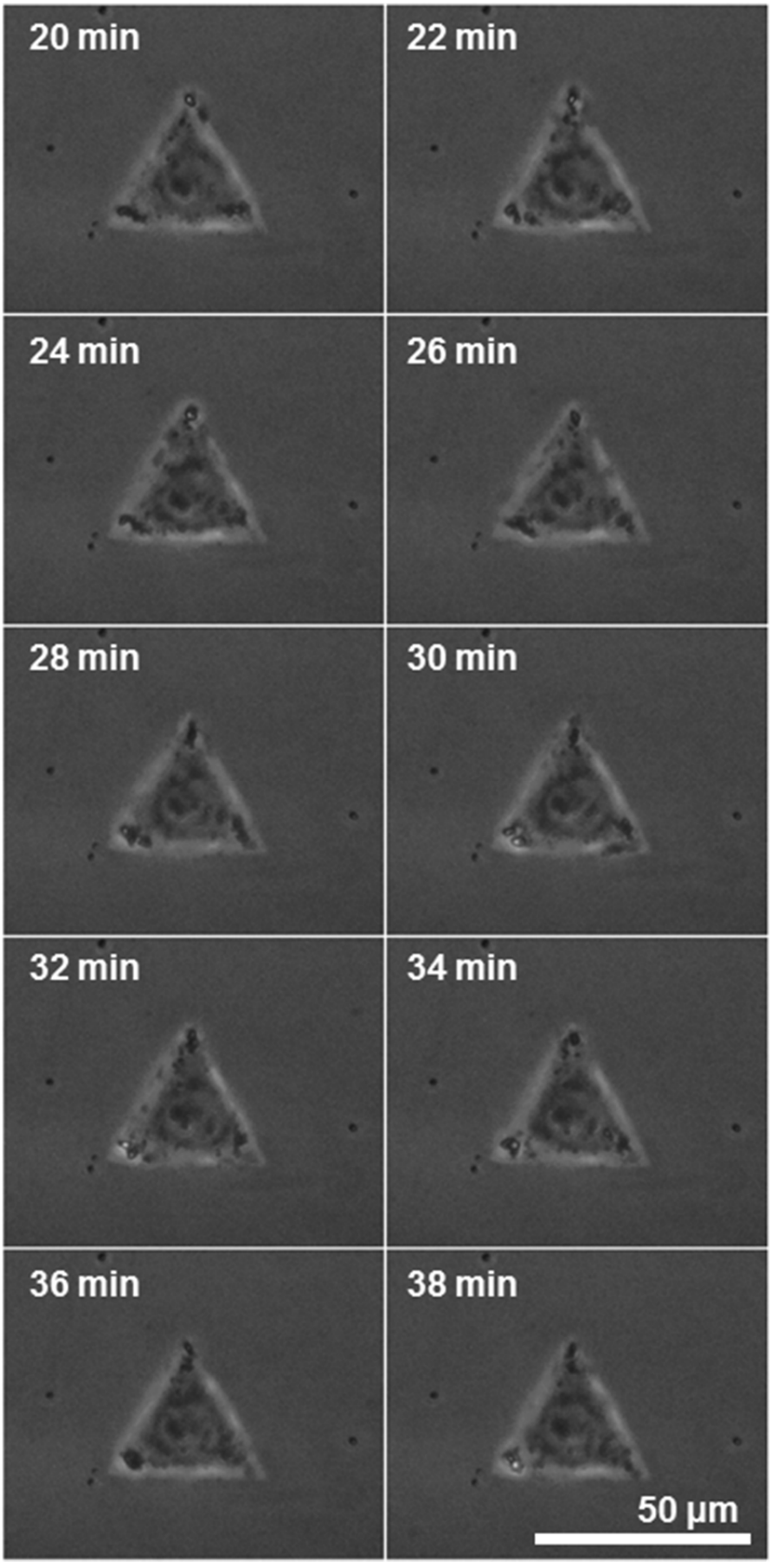


Figure S8: Micrographs taken from time-lapse imaging of a PatuT cell constrained to a circular island (adhesive area: 900 μm^2).


0 min
2 min
4 min
6 min
8 min
10 min
12 min
14 min
16 min
18 min
50 μm


20 min
22 min
24 min
26 min
28 min
30 min
32 min
34 min
36 min
38 min
50 μm

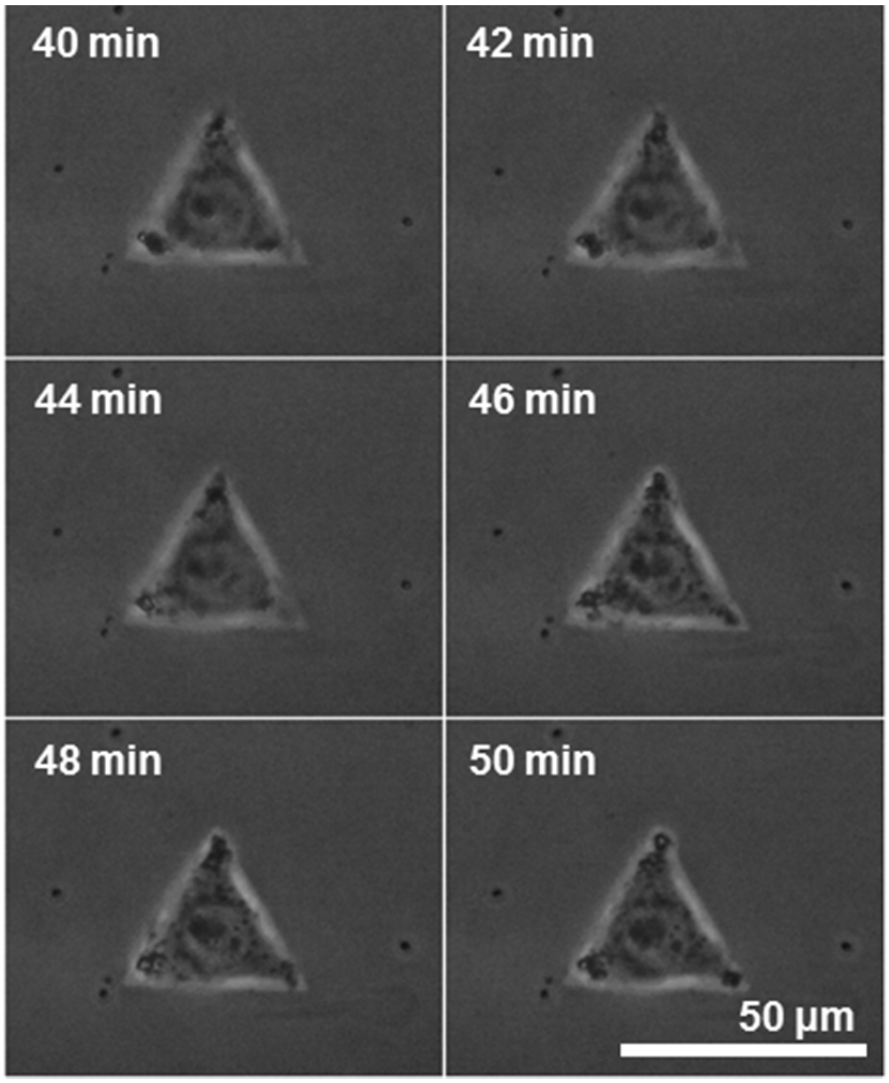


Figure S9: Micrographs taken from time-lapse imaging of a PatuT cell constrained to a triangular island (adhesive area: 900 μm^2)

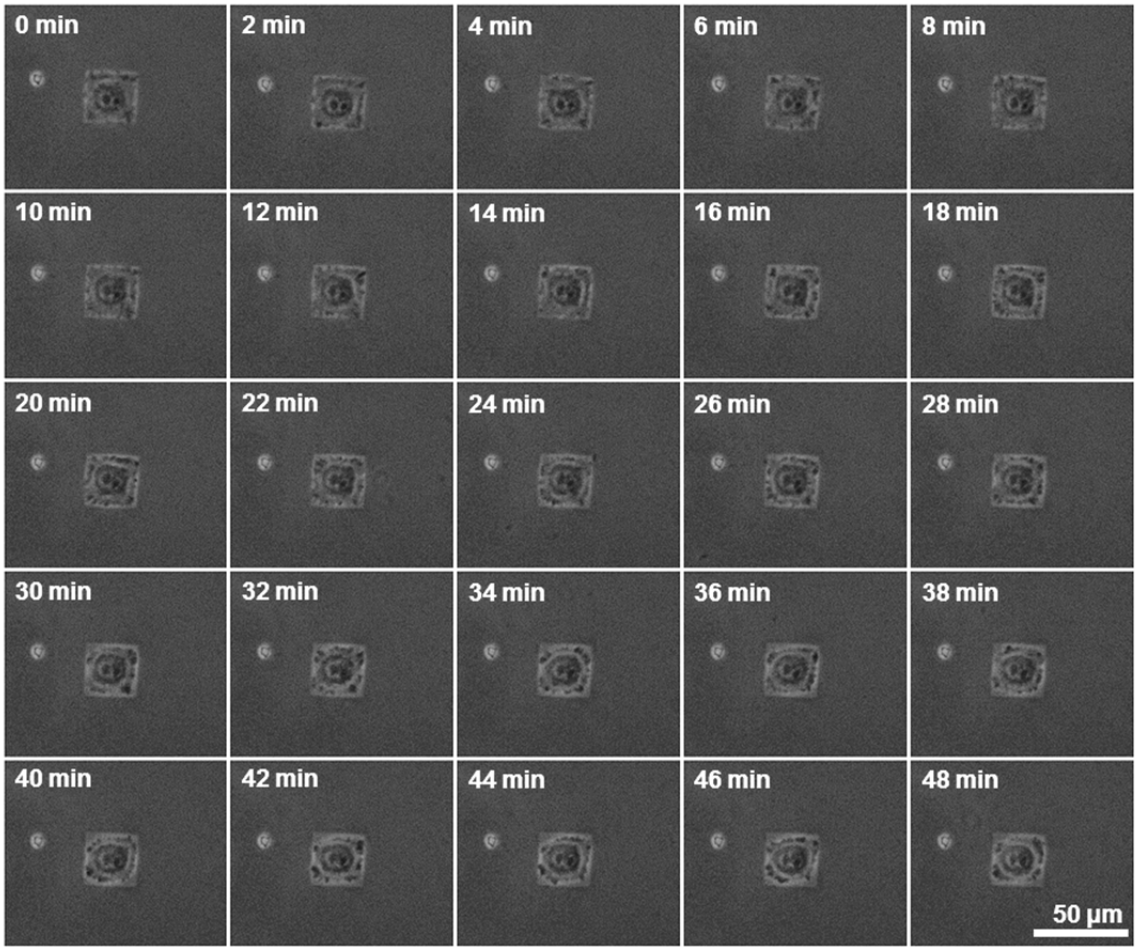


Figure S10: Micrographs taken from time-lapse imaging of a PatuT cell constrained to a square island (adhesive area: 900 μm^2).

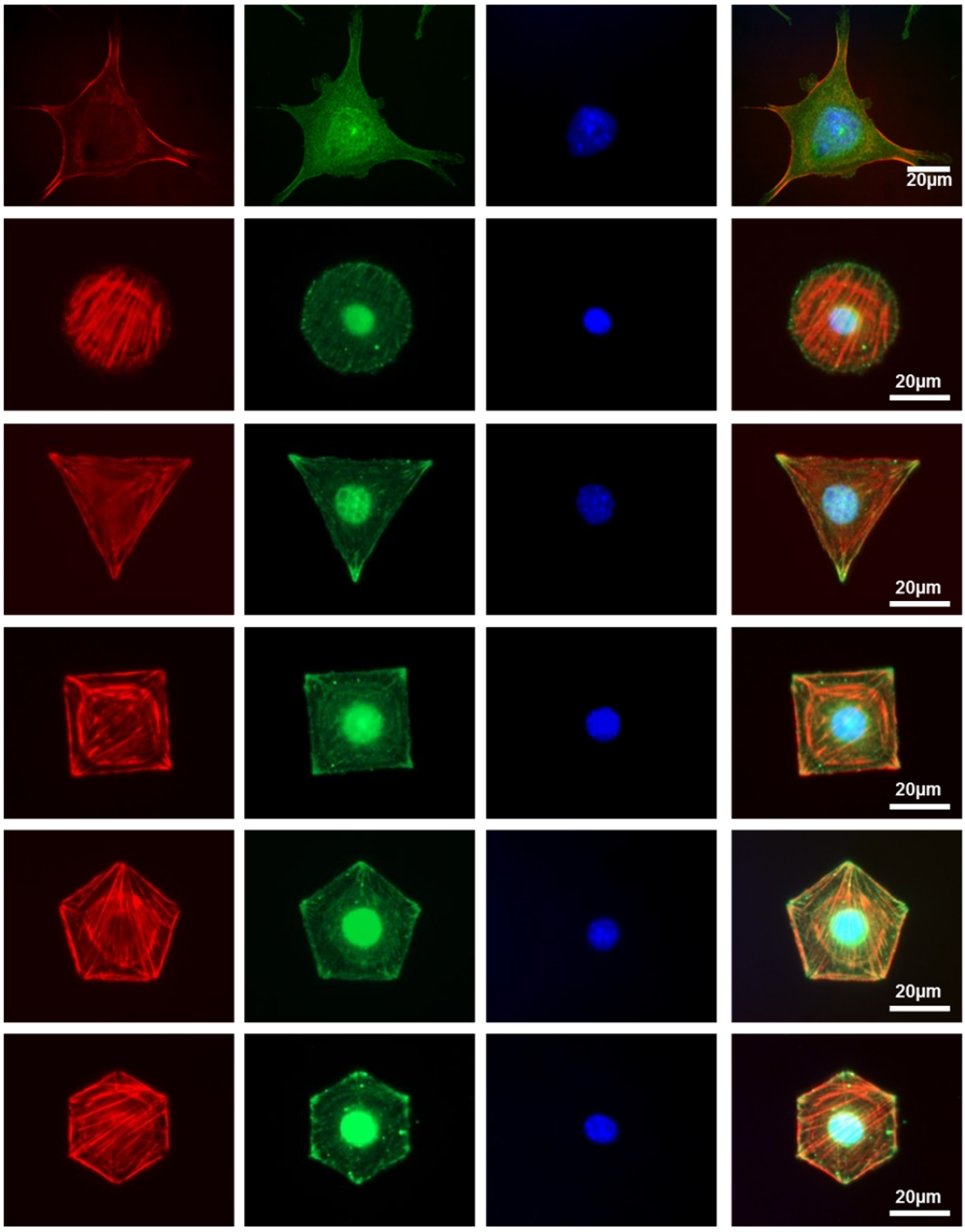


Figure S11: Fluorescence microscopy images of single NIH 3T3 cells on a non-patterned substrate (SAM of ODT) and on adhesive islands with different geometries (adhesive area: 1600 μm^2) stained for actin (red), paxillin (green) and nuclei (blue) after culture for 6h.

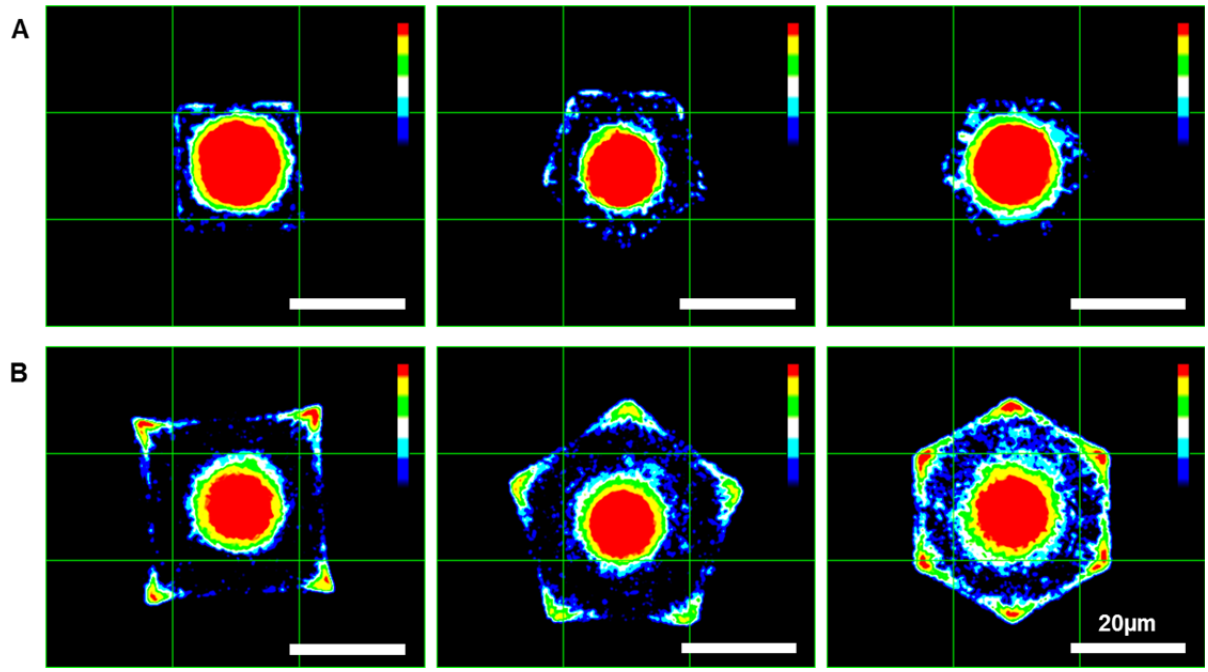


Figure S12: Fluorescence heatmaps of >15 single PatuT (A) and NIH 3T3 (B) cells with different geometries (area: 900 µm^2 and 1600 µm^2, respectively) stained for paxillin as a quantitative measure of focal adhesions.

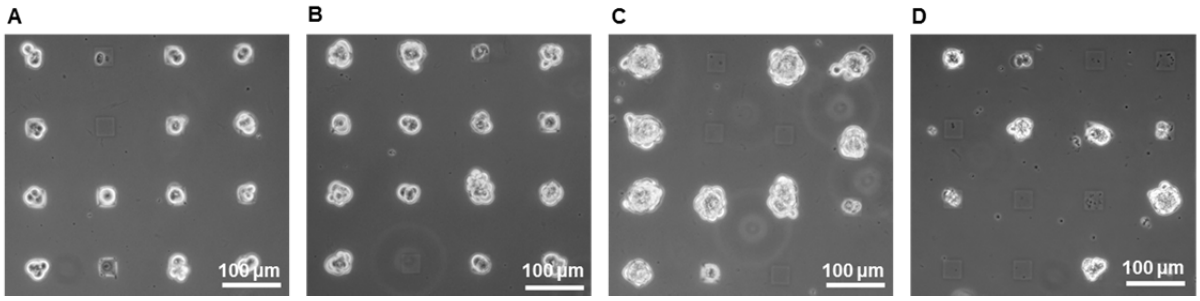


Figure S13: Light microscopy images of NIH 3T3 fibroblast cells on patterned PAAm brushes with square islands (adhesive area: 1600 µm^2) demonstrating the pattern stability over a period of 15 days (A: day 2, B: day 4, C: day 6 and D: day 14).

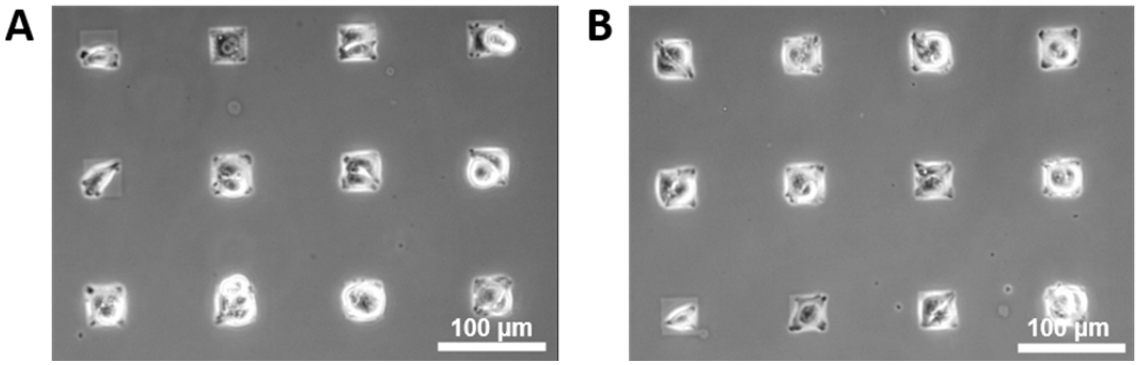


Figure S14: Light microscopy images of NIH 3T3 fibroblast cells on patterned PAAm brushes (adhesive area: 1600 µm^2) stored under ambient conditions before cell culture (A: 3 month and B: 6 month).

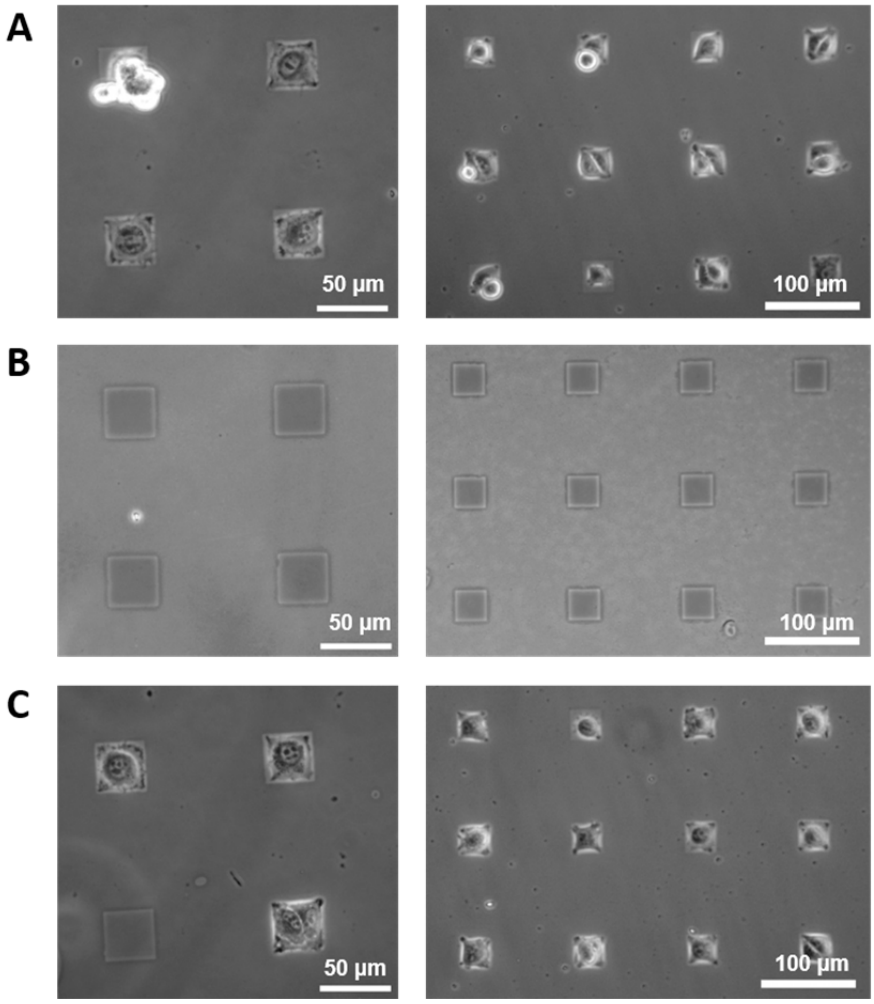


Figure S15: Light microscopy images of PatuT cells on PAAm brush patterns (adhesive area: 900 µm^2). A: pattern stability after 6 hours incubation. B: pattern without cells in air after trypsin treatment and C: pattern stability after the second cell seeding and 6 hours incubation**.**

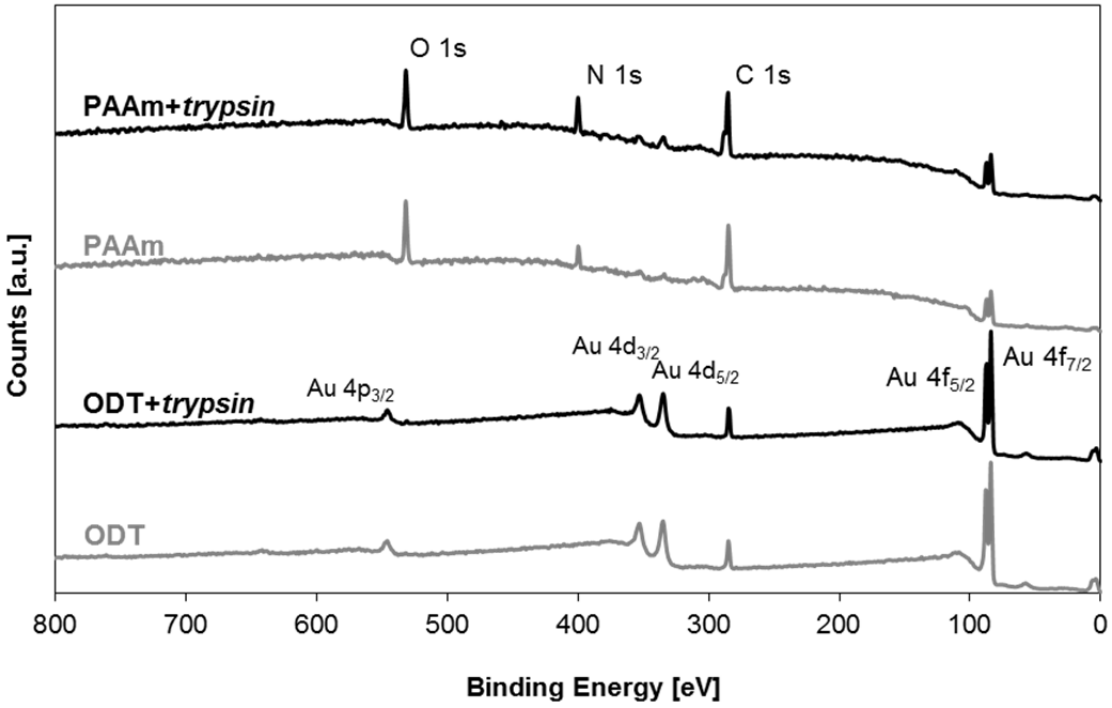


Figure S16: XPS spectra of ODT and PAAm brushes before and after treatment with trypsin.

Table S4: Elemental composition in atom-% as determined by XPS of ODT and PAAm brushes on gold coated substrates before and after treatment with trypsin (Gibco, Life Technologies).

Sample	XPS atomic concentration [atom-%]				Ratio	
	C 1s 285 eV	**N 1s** 400 eV	**O 1s** 532 eV	**S 2p** 162 eV	**N/C**	**O/C**
PAAm	64.9	17.2	17.9	-	0.26	0.30
PAAm-trypsin	65.2	16.4	18.4	-	0.25	0.28
ODT	96.5	-	-	3.5	-	-
ODT-trypsin	93.8	-	2.9	3.2	-	0.03
Trypsin*					0.28	0.54

Video S1: Time-lapse video [60min; 4 fps] of PatuT cells settling on square islands (adhesive area: 900 μm^2).

Video S2: Time-lapse video [50min; 4 fps] of the proliferation of PatuT cells on square islands (adhesive area: 900 μm^2).

Video S3 circle: Time-lapse video [50min; 4 fps] of a PatuT cell on a circular island (adhesive area: 900 μm^2).

Video S4: Time-lapse video [50min; 4 fps] of a PatuT cell on a triangular island (adhesive area: 900 μm^2).

Video S5: Time-lapse video [50min; 4 fps] of a PatuT cell on a square island (adhesive area: 900 μm^2).

References

[1] Lilge, I., Schönherr, H. Synthesis and Characterization of Well-Defined Ligand-Terminated Block Copolymer Brushes for Multifunctional Biointerfaces. *Polymer* **2016**, *98*, 409-420.

[2] Lilge, I., Schönherr, H. Covalently Cross-Linked Poly(acrylamide) Brushes on Gold with Tunable Mechanical Properties via Surface-Initiated Atom Transfer Radical Polymerization. *Eur. Polym. J.* **2013**, *49*, 1943–1951.

[3] Ma, H.; Hyun, J.; Stiller, P.; Chilkoti, A. "Non-Fouling" Oligo(ethylene glycol)-functionalized Polymer Brushes Synthesized by Surface-Initiated Atom Transfer Radical Polymerization. *Adv. Mater.* **2004,** *16*, 338–341.

[4] Jones, D. M., Brown, A. A., Huck, W. T. S. Surface-Initiated Polymerizations in Aqueous Media: Effect of Initiator Density. *Langmuir* **2002**, *18*, 1265–1269.

[5] DiMilla, P. A., Folkers, J. P., Biebuyck, H. A., Haerter, R., Lopez, G. P., Whitesides, G. M. Wetting and Protein Adsorption on Self-Assembled Monolayers of Alkanethiolates Supported on Transparent Films of Gold. *J. Am. Chem. Soc.* **1994**, *116*, 2225–2226.