Electronic Supplementary Information

Impact of structure dimensions on initial bacterial adhesion

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periodicity	surface	advancing [°]	±	receding [°]	±
0 nm	APTES	35	28	0	0
5000 nm	APTES	35	23	0	0
2000 nm	APTES	53	20	0	0
1000 nm	APTES	48	15	0	0
750 nm	APTES	34	18	0	0
500 nm	APTES	25	12	0	0
0 nm	SU8	81	3	55	3
5000 nm	SU8	92	11	41	5
2000 nm	SU8	94	3	34	2
1000 nm	SU8	87	4	38	3
750 nm	SU8	86	4	46	6
500 nm	SU8	86	5	45	5
0 nm	TAF	120	4	109	4
5000 nm	TAF	122	8	85	21
2000 nm	TAF	118	9	55	16
1000 nm	TAF	119	8	86	22
750 nm	TAF	119	3	103	6
500 nm	TAF	120	6	97	18

Table S1. Contact angle data for funtionalized APTES-, SU8- and TAF-hole structures.

periodicity	structure	advancing [°]	±	receding [°]	±
0 nm	none	81	3	55	3
5000 nm	holes	92	11	41	5
1000 nm	holes	87	4	38	3
500 nm	holes	86	5	45	5
5000 nm	posts	86	2	30	5
1000 nm	posts	83	2	42	6
500 nm	posts	81	5	28	9
5000 nm	lines [o)]	104	10	47	4
1000 nm	lines [o)]	98	5	35	8
500 nm	lines [o)]	88	3	36	10
5000 nm	lines [p)]	78	1	43	6
1000 nm	lines [p)]	74	1	34	4
500 nm	lines [p)]	78	2	34	12

Table S2. Contact angle data of SU8 hole, post and line structures. [o)] angle at the wetting front orthogonal to the lines. [p)] angle at the wetting front parallel (along) the lines

Surface + treatment	C (1s)	O (1s)	N (1s)	Si (2p)
SU8	82.9	17.1		
SU8 + 60 s	78.9	21.1		
SU8 + 120 s	77.0	23.0		
SU8 + 240 s	76.9	23.1		
SU8 + APTES	77.5	19.6	1.2	1.7
SU8 + 60 s + APTES	54.9	29.8	7.0	8.3
SU8 + 120 s + APTES	51.6	32.1	7.3	9.0
SU8 + 240 s + APTES	61.5	25.9	6.2	6.4

Table S3. XPS data of relative amount of elements on SU8 surface. The comparison of different treatment times with O_2-Plasma were used to find an optimal activation before etching processes disturb the functionalization of the photo resist SU8. The optimal treatment time turned out to be 120 s (red box).

Sample preparation

For fabrication of the one (lines) and two (posts and holes) dimensional structures, the photoresist SU-8 (2) (MicroChem Corp.) was used. Polished silicon wafers were sonicated in 2-propanol for 15 min. Subsequently, the SU-8 was spin-coated with 3000 rpm for 30 s. Samples were prebaked for 4 min at 95 °C and then exposed to the interference patterns. The interference pattern was applied with a high power pulsed Nd:YAG laser system (Quanta-Ray PRO, Spectra Physics) with a pulse duration of 10 ns and a lens setup (**Figure S3**). After passing the beam shaping lens and mask, the output beam was divided into two or three laser beams for the fabrication of the line-like and post-like patterns, respectively. The mirrors were positioned to ensure that the laser beams interfere on the samples surface. Hole-like structures were fabricated using a double exposure process consisting of irradiation of the sample with a line-like interference pattern followed by a rotation of the sample with an angle of 60° and a second irradiation step. For each geometrical pattern the spatial period was set to vary between 500 nm and 5000 nm by adjusting the intercepting angle α between the beams. For the two and three beam configuration, it is given by Eq. 1 and 2, respectively (with λ being the laser wavelength).

$$\Lambda = \frac{\lambda}{2 \cdot \sin(\alpha/2)} \quad (1)$$

$$\Lambda = \frac{\lambda}{\sqrt{3} \cdot \sin(\alpha/2)} \quad (2)$$

Angles (α) of 20.7, 10.2 and 2.0° permitted to fabricate 500, 1000 and 5000 spatial periods for the line-like and hole-like arrays. For the fabrication of the post-like patterns, the angles were set to 24.2°, 11.8° and 2.35° so that 500, 1000 and 5000 nm spatial periods can be respectively obtained. Irradiation fluence between 4 and 10 mJ/cm² was chosen to obtain homogenous periodic patterns. Afterwards, the irradiated substrates were post-baked at 95 °C and developed for 1 min using PGMEA developer (MicroChem Corp.). Finally, the samples were washed in ethanol for 30 s.

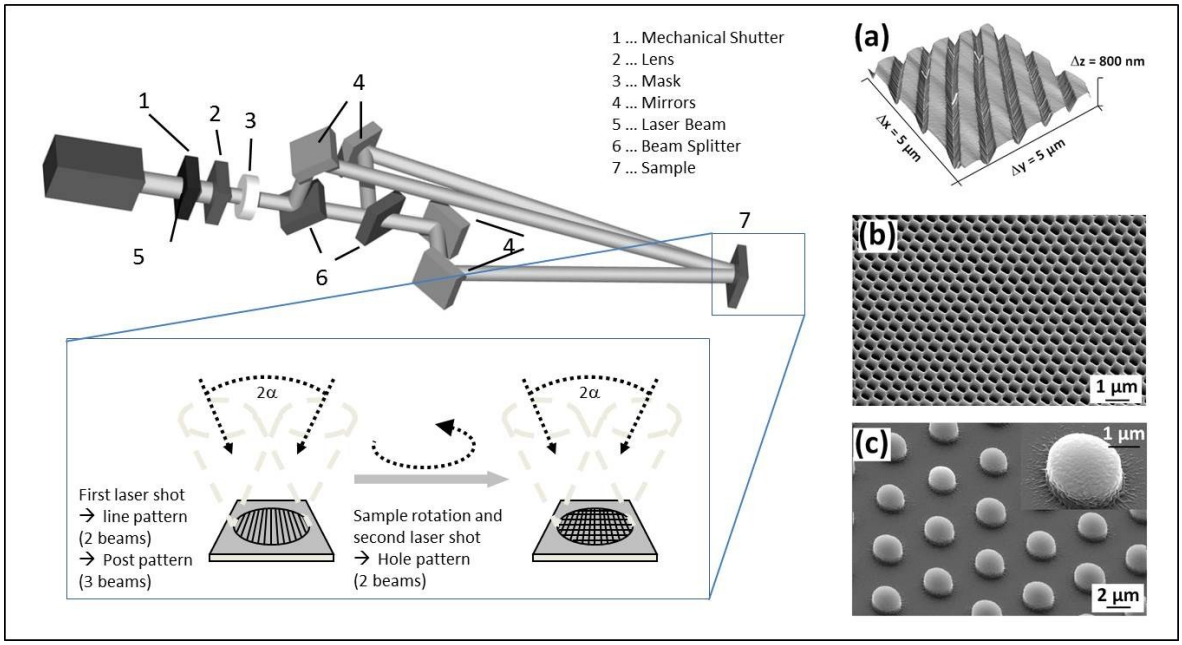

Figure S1. LIP setup. (a) AFM image of a Λ = 1 µm line structure, (b) SEM image of Λ = 500 nm hole structure; (c) SEM image of Λ = 5 µm post structure.

Functionalization and wetting properties

For cleaning and hydrophilization, samples were first sonicated in Ethanol for 10 min and then treated in a residual air plasma cleaner (Harrick Scientific Corp. PDC-002) for two minutes. The evaluation of appropriate activation time was done in 1 - 4min time intervals and analysis of the surface by subsequent x-ray photoelectron spectroscopy. We used an Amicus Spectrometer from Kratos Analytical (UK) with a non-monochromatic Mg K_α X-ray source operated at 240 W and 8 kV. The results are shown in supplemental **Table S3**. Subsequently, samples were functionalized with (3-Aminopropyl)triethoxysilane by immersion in a solution of isopropanol:water:silane (200:10:1) for 90 min at room temperature. Afterwards the samples were rinsed three times in pure isopropanol, dried with nitrogen and tempered for 2 h at 90°C. For hydrophobization, samples were first sonicated for 10 min in Ethanol and then dip-coated with the amorphous fluorpolymer TAF (Dupont®; 1:100 diluted with FC77, Dupont®). TAF layer thickness was determined by ellipsometric measurements (M-2000V, J.A. Woolam Co.) on unstructured SU8 substrates which resulted in 13 ± 4 nm. For each sample type, at least four measurements of the advancing and receding contact angle (OCA30 from Dataphysics) were performed by dispensing and re-dispensing 10 µl of degased MilliQ water at a velocity of 0.3 µl/s.

Bacterial assays

Staphylococcus epidermidis (strain ATCC 12228, non-piliated, USA) and *Escherichia coli* (strain K12 DH5, non-piliated, DZMS, Germany) were routinely grown at 37 °C in Luria-Bertani (LB) medium. For the bacterial assays, overnight cultures were centrifuged at 3000 g for 5 min. The supernatant was removed and the pellet was resuspended in phosphate buffer solution (PBS). This washing step was repeated three times. Afterwards the cell density was adjusted to an OD_{600} of 0.75 or 0.0075 (corresponding to an absolute cell number of $6 \cdot 10^8$ ml^{-1} or $6 \cdot 10^6$ ml^{-1}). Subsequently, the structured samples were incubated in the bacterial solutions at 37°C for 2 min (OD600 = 0.75) or 24 h (OD600 = 0.0075) in an orbital shaker (60 rpm with an orbit of 19 mm, VWR Model 3500I). After the defined time periods the samples were fixed in 8% Formol for 15 min without an intermediate rinsing for the 2 min assay and with rinsing in PBS for the 24 h assay. For two minutes exposure cells are only loosely bound and washing removes many of them, but it was the intention to observe all firstly arrived cells on the surface. For the 24h exposure a washing step by rinsing with PBS was intentionally used to remove loosely bound cells, in order to observe the irreversibly attached bacteria on the substrates. After fixation the samples were washed with PBS, than washed with MilliQ and finally carefully dried with nitrogen.

For fluorescence imaging, bacterial cells were stained with DAPI (4',6-diamidino-2-phenylindole, dihydrochloride, 1:10000, Invitrogen) between fixation and the washing steps. For SEM imaging, samples were sputter-coated with a 10 nm gold layer (BALZERS SCD 050 Sputter Coater).

Image acquisition and analysis

DAPI-stained samples were imaged with Zeiss Axiotech Optical Microscope (20x and 50x air lens) using a DAPI filter. Gold-sputtered samples were imaged with a Scanning Electron Microscope (Zeiss Gemini Ultra Plus) at 3 kV. Images were analyzed using Image J (v. 1.45, Wayne Rasband, NIH USA). Three independent experiments with at least three samples per condition were performed. The number of cells adhering to the structured substrates was quantified within at least 10 frames per sample type. There was no statistically significant difference between fluorescence imaging and electron microscopy. Thus, both data were combined and prepared as whisker plots.