Micro-Topography Influences Blood Platelet Spreading - Supporting Information

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Figure S1: Micrographs showing the coating quality. The substrates have been coated as described in section Substrate Preparation. a) Fluorescence and phase contrast images of a microcontact-printed (structured, selectivelycoated) substrate. b) Fluorescence and phase contrast images of a fibrinogencoated (structured, completely coated) substrate.



Figure S2: Pictures of different steps in image processing. a) Original TxRedimage of actin structures of the cell, inverted. b) Detected outlines by canny algorithm (in MATLAB). c) Binarized image (in ImageJ) after manually closing outlines detected by the canny algorithmus.



Figure S3: Histograms showing the cell area of platelets on structured substrates with an array of 2.8 μ m wide holes. Data for three different substrate types is shown: (top) on flat completely fibrinogen-coated substrates; (center) on topographically structured, completely fibrinogen-coated substrates; (bottom) on topographically structured, selectively fibrinogen-coated substrates. The spreading area of the cells is similar for all three substrate types and corresponds nicely with the spreading area found for cells on structured substrates with an array of 2.1 μ m wide holes.



Figure S4: Histograms showing the ratio of the cell perimeter U_{cell} to the perimeter of an ellipse $U_{ellipse}$ that has the same area as the cell for platelets on structured substrates with an array of 2.8 μ m wide holes. The ratio increases from top to bottom - similar to what is found for cells on structured substrates with an array of 2.1 μ m wide holes.



Figure S5: Histograms showing the difference in average membrane curvature of platelets on structured substrates with an array of 2.8 μ m wide holes. (top) on completely coated, topographically structured substrates as compared to platelets on flat substrates; the difference values (structure-flat) Δ percentage are shown; the inset shows the different curvatures along the contour of the holes. (bottom) on selectively coated, topographically structured substrates as compared to platelets on flat substrates.



Figure S6: Confocal stack (see movie M1) of a cell on a completely coated substrate with an array of approximately $3.0 \,\mu\text{m}$ wide holes. The z-stack has been recorded with an inverted motorized research microscope (IX81, Olympus, Hamburg, Germany) upgraded with a Fluoview 1000 confocal unit (Olympus, Hamburg, Germany) equipped with an 100x oil-immersion phase contrast objective and the confocal software FV-10-ASW 4.0 (Olympus, Hamburg, Germany). The fibrinogen-coating of the substrate is shown in gray and the actin structure of the platelet in red. The field of view consists of $512 \times 512 \,\text{px}$ corresponding to $12.264 \times 12.264 \,\mu\text{m}$ (here cropped to $480 \times 480 \,\text{px}$). Δz has been set to $250 \,\text{nm}$. The z-stack starts above the cell and reaches planes inside the hole as it progresses. While progressing inside the hole, two lamellipodia emerge, indicating that the cell reaches inside the holes and attaches those lamellipodia to the bottom of the holes.