Supplementary Information

A Simple Nanoscale Interface Directs Alignment of a Confluent Cell Layer on Oxide and Polymer Surfaces

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Reference Fig. S1. Cell modeled as an ellipse used to find cell aspect ratio (ratio of width to length) and angle of orientation, θ .



Fig. S2. XP spectra of 4-modified (red) and unmodified SiO₂/Si. (A) Survey spectrum; (B) Zr(3d) region, deconvoluted best-fit components in blue; (C) Zr(3p) region.



Fig. S3. (A) Survey XP spectra of nylon 6,6 before (black) and after (red) surface modification to form 4; (B) P(2p) region for 4-modified and unmodified nylon 6,6. Note appearance of Zr(3d) peak at 183.5 eV and the P(2p) at 134.5 eV



Fig. S4. (A) Survey XP spectra of PEEK before (black) and after (red) surface modification to for 4, (B) P(2p) region for 4-modified and unmodified PEEK. Note appearance of Zr(3d) peak at 184.3 eV and the P(2p) at 135.1 eV.



Fig. S5. (A) Survey XP spectra of PET before (black) and after (red) surface modification to form **4**. (B) P(2p) region for **4**-modified and unmodified PET. Note appearance of Zr(3d) at 184.3 eV and the P(2p) at 135.0 eV.



Fig. S6. SEM images and EDS of nylon 6,6 (A-C, scale bar = 100 μ m), PET (D-F, scale bar = 200 μ m), and PEEK (G-I, scale bar = 100 μ m) after 60 X 30 templating, 5 min exposure to 1, and modification with 4; (J) PEEK surface functionalized with 4 following a 3 min exposure to 1. SEM images in the left column, EDS maps of Zr in the middle column (magenta), and maps of P in the right column (cyan).



Fig. S7. (A) AFM of a 40 X 30 4-stipe edge on SiO_2/Si and line segment for height plot; (B) height plot from A for 4-stipe edge, 4-stripe is approximately 40 nm high; (C) AFM of a 60 X 30 4-stripes on PET and line segment for height plot; (D) height plot from C for 4-stripeedge, 4-stripe is approximately 70 nm high.



Fig. S8. Optical images of glass (A-B) and silicon (C-D) modified with 30 X 30 patterns of **2**. (A) Glass surface day 0 (before immersion in serum containing medium); (B) 18 days after immersion; (C) silicon surface day 0; (D) 18 days after immersion. Note the intact stripe edges.



Fig. S9. XP spectra of 30 X 30 **2** stripes on silicon (A) and glass (B) before (day 0) and after (day 9 and 18) immersion in serum containing medium. Note the appearance of the N(1s) at 339.5 eV (A) and 399.4 eV (B), and the attenuation of the Zr(3d) at 182.4 eV (A) and 182.3 eV (B), which is no longer visible on (B) after 9 days.



Fig. S10. XP spectra of the C(1s) peak on 30 X 30 2 stripes on silicon before (day 0) and after (days 9 and 18) immersion in serum containing medium. Note the appearance of higher binding energy shoulders after immersion, supporting adsorption of protein species to the surface.



Fig. S11. AFM images of silicon (A-C) and glass (D-F) coated with 30 X 30 patterns of **2**. (A) Day 0 stripe (before immersion in serum containing medium), stripe height = 12 nm;^a (B) 9 days after immersion, height = 22 nm; (C) 18 days after immersion, height = 20 nm; (D) day 0, height = 12 nm; (E) 9 days after immersion, height = 30 nm; (F) 18 days after immersion, height = 50 nm. ^aRelative to the uncoated region of the substrate.



Fig. S12. SEM image (A, scale bar = 100 μ m), and complementary EDS maps of Zr (B) and N (C) of a **2**-patterned SiO₂/Si surface after 3 days in serum containing medium. Note the visible ZrO₂ pattern but the evenly distributed N signal supporting that adsorbed protein is not localized.



Fig. S13. NIH 3T3 Fibroblasts spread on 4-modified patterns on SiO₂/Si after 24 hr in culture. (A) 10 X 10, (B) 20 X 20, (C) 10 X 20, (D) 20 X 30, (E) 30 X 10, (F) 40 X 30, (G) 50 X 30, (H) 60 X 30, (I) 4-modified, unpatterned control. Images are of actin stain, scale bar = $100 \mu m$. Insets are increased brightness images of the nuclei stain.



Fig. S14. Box plots of cell aspect ratio (A) and angle of orientation (B) for NIH 3T3 fibroblasts after 24 hr in culture on 4-patterned and 4-unpatterned, control surfaces of SiO_2/Si . The square point is the mean of the data set, the box edges are the 75th and 25th percentiles, the horizontal line in the box is the median, and the bars are the 95th and 5th percentiles. Each box represents at date set of 100 measurements.



Fig. S15. NIH 3T3 Fibroblasts spread on unpatterned 4-modified SiO₂/Si after 24 hr (A) and 3 days in culture (B).Images represent actin stain, scale bar = $100 \mu m$.

Table S1. Summary of elastic moduli measurements for unmodified, **2**-modified, and **4**-modified polymers. A one-way ANOVA was performed on each data set (n = 5) with significance at $\alpha < 0.05$. Moduli are reported as average \pm standard deviation in GPa. The bottom row of the table shows the resulting *p*-value from the ANOVA.

Material	PET	PEEK	Nylon 6,6
Unmodified	3.8 ± 0.2	1.8 ± 0.2	1.6 ± 0.2
2-modified	4.1 ± 0.5	1.5 ± 0.1	1.5 ± 0.1
4-modified	3.8 ± 0.5	1.7 ± 0.3	1.5 ± 0.2
ANOVA	<i>p</i> = 0.367	<i>p</i> = 0.136	p = 0.265