Supplementary data

Fibronectin adsorption on oxygen plasma-treated polyurethane surfaces modulates endothelial cell response

Ruben Daum[1,2,3§], Ivana Mrsic[4§], Johanna Hutterer[4], Achim Junginger[4], Svenja Hinderer[1], Alfred J. Meixner[4,5], Günter Gauglitz[4], Thomas Chassé[4,5] and Katja Schenke-Layland[1,2,3,6,7] *

[1] NMI Natural and Medical Sciences Institute at the University of Tübingen, Markwiesenstr. 55, 72770 Reutlingen, Germany

[2] Department of Women's Health, Research Institute for Women's Health, University of Tübingen, Silcherstr. 7/1, 72076 Tübingen, Germany

[3] Department of Bioengineering, University of Tübingen, Silcherstr. 7/1, 72076 Tübingen, Germany

[4] Institute of Physical and Theoretical Chemistry, University of Tübingen, Auf der Morgenstelle 18, 72076 Tübingen, Germany

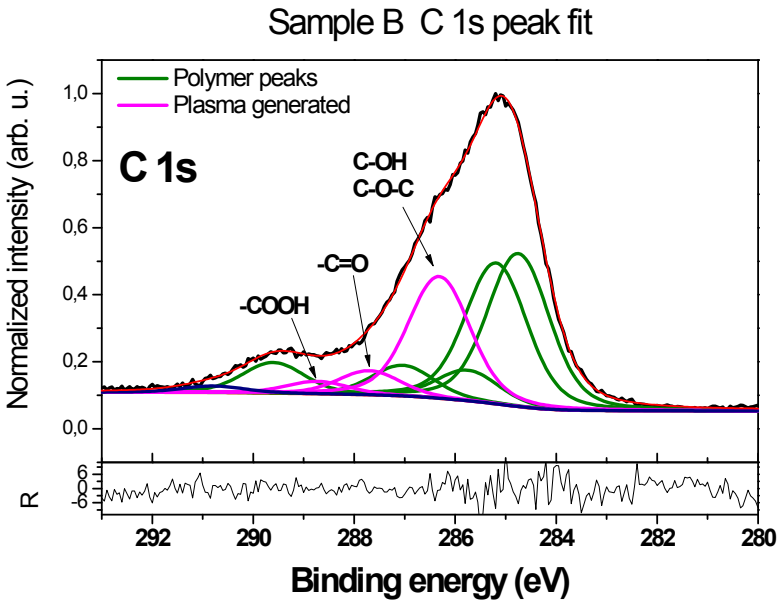
[5] Center for Light-Matter Interaction, Sensors & Analytics (LISA+) at the University of Tübingen, Auf der Morgenstelle 18, 72076 Tübingen, Germany

[6] Cluster of Excellence iFIT (EXC 2180) "Image-Guided and Functionally Instructed Tumor Therapies", University of Tübingen, 72076 Tübingen, Germany

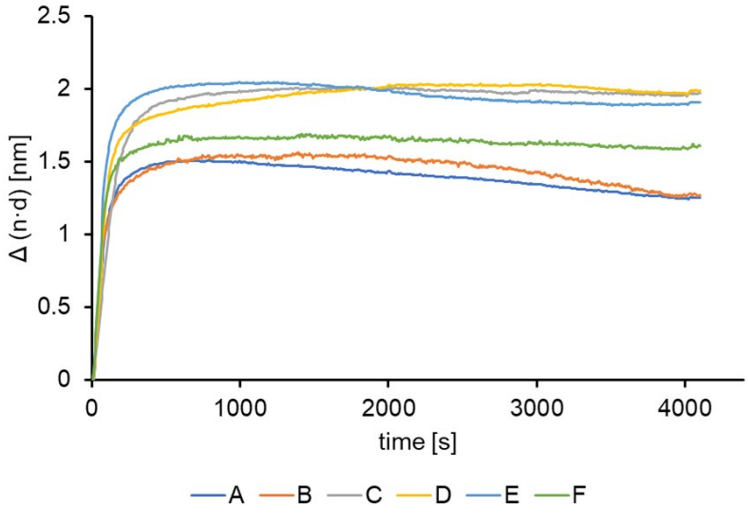
[7] Department of Medicine/Cardiology, Cardiovascular Research Laboratories, David Geffen School of Medicine at UCLA, Los Angeles, CA 90095, USA

*Correspondence: katja.schenke-layland@uni-tuebingen.de; Tel.: +49-707-1298-5205; www.schenke-layland-lab.de

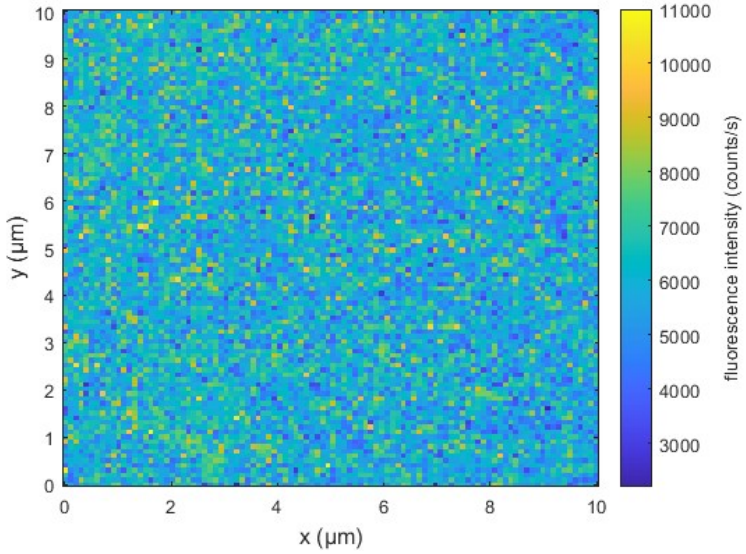
[§]These authors contributed equally to this work



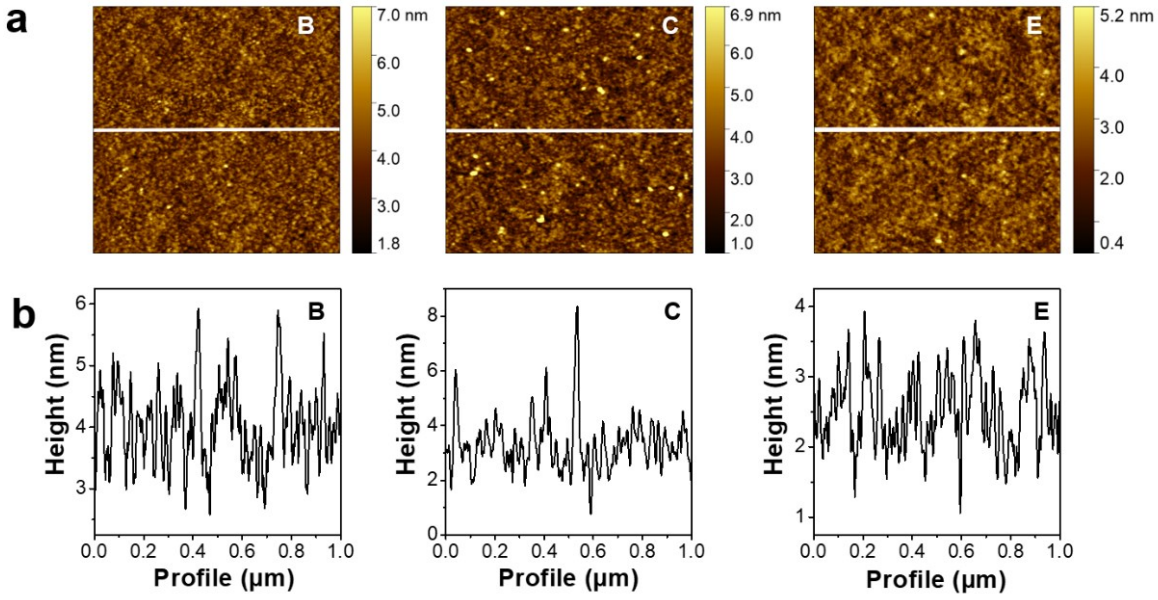
Supplementary Figure 1. XPS peak fit example of sample B (GP-FWHM = 1.205 eV; LP-FWHM = 0.42 eV; FWHM = 1.445 eV). Spectra were collected with 20 eV pass energy, 0.2 s dwell time, and 0.05 eV step size. The pink peaks represent the plasma generated functional groups, except C-O-C which is part of the polyurethane structure. The green peaks come from the polyurethane carbon groups (PU: O(O**C**)N at 289.6 eV; **C**–O(OC)N at 287.1 eV); C–O–C at 286.3 eV; O(OC)N–**C** at 285.8 eV; **C**–C at 285.1 eV; **C**=C at 284.7; plasma-induced: **C**–OH at 286.3 eV; –**C**=O at 287.7 eV; –**C**OOH at 288.8 eV).



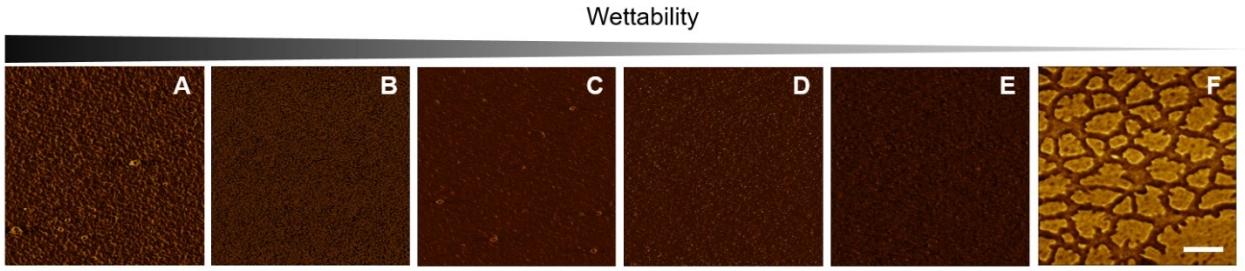
Supplementary Figure 2. Binding curves of 10 µg/ml fibronectin on surfaces A, B, C, D, E and F measured with Reflectometric Interference Spectroscopy.



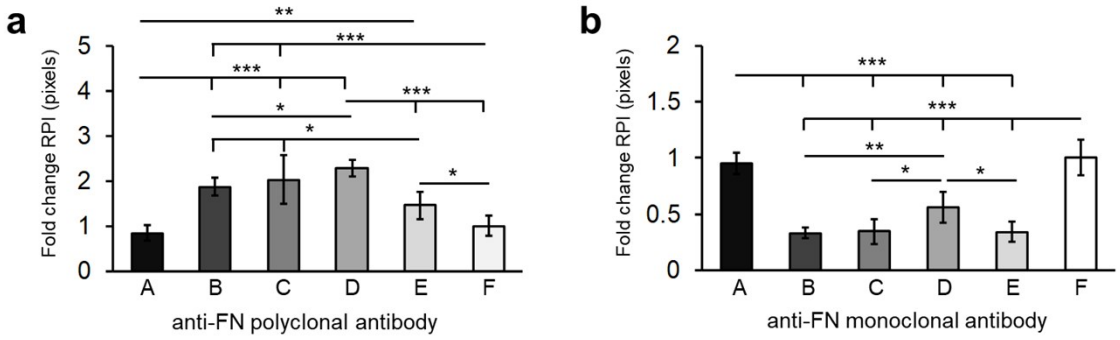
Supplementary Figure 3. Exemplary laser confocal scanning microscopy image of DY-490 labeled FN coated on sample F. The samples showed homogeneous fluorescence signals. Pixel size is 100 nm.



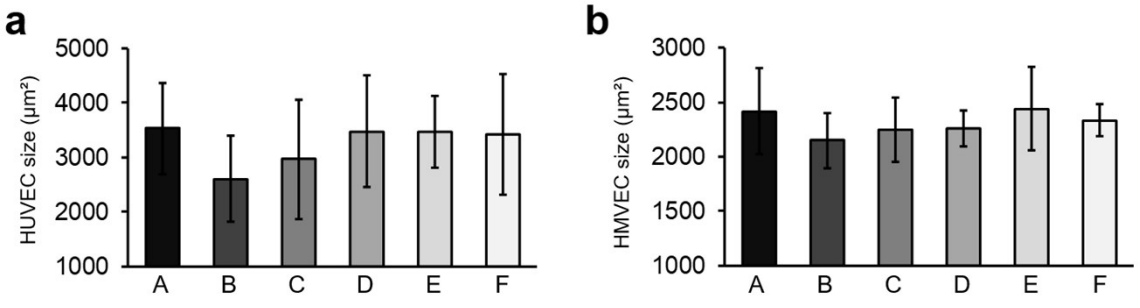
Supplementary Figure 4. AFM (**a**) height contrast images of plasma treated surfaces with FN coating. The white line represents the region of the extracted height profiles. (**b**) Extracted height profile lines of the FN-coated polymer surfaces showcasing the surface structuration.



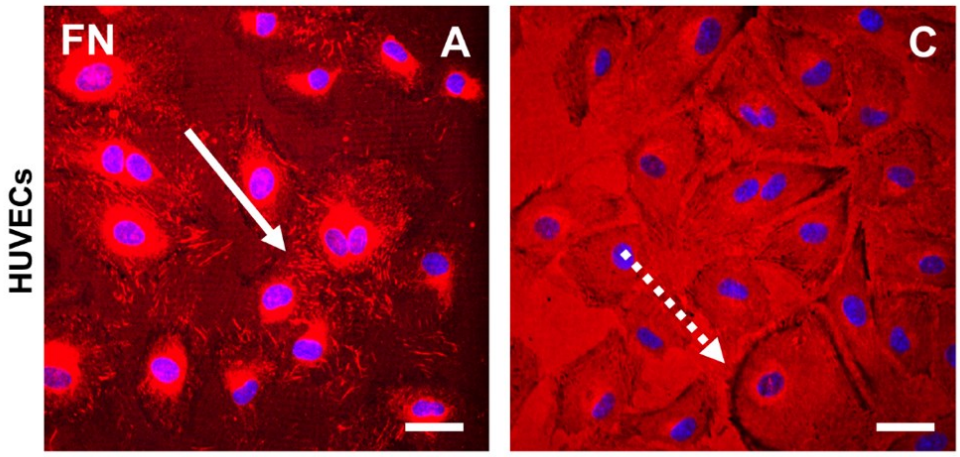
Supplementary Figure 5. AFM material contrast images of the sample surfaces after FN coating. The scale bar equals 200 nm.



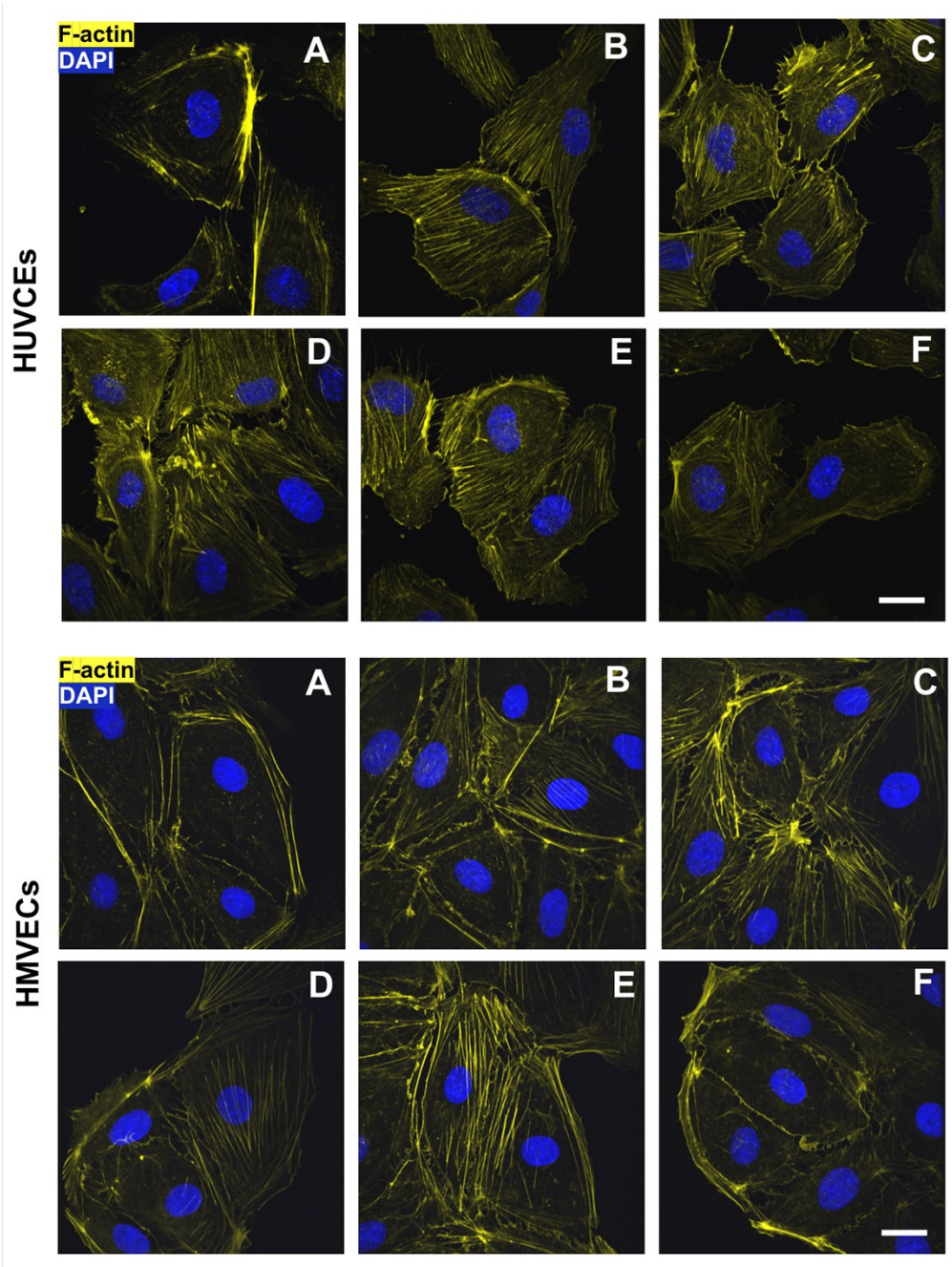
Supplementary Figure 6. (**a**) Semi-quantitative pixel intensity analysis of the polyclonal anti-FN immunofluorescence staining. Data is shown relative to surface F. (**b**) Monoclonal anti-FN immunofluorescence staining against the cell binding domain. Data is shown relative to surface F. One-way ANOVA, n=3, *p<0.05, **p<0.01, ***p<0.001; RPI = relative pixel intensity.



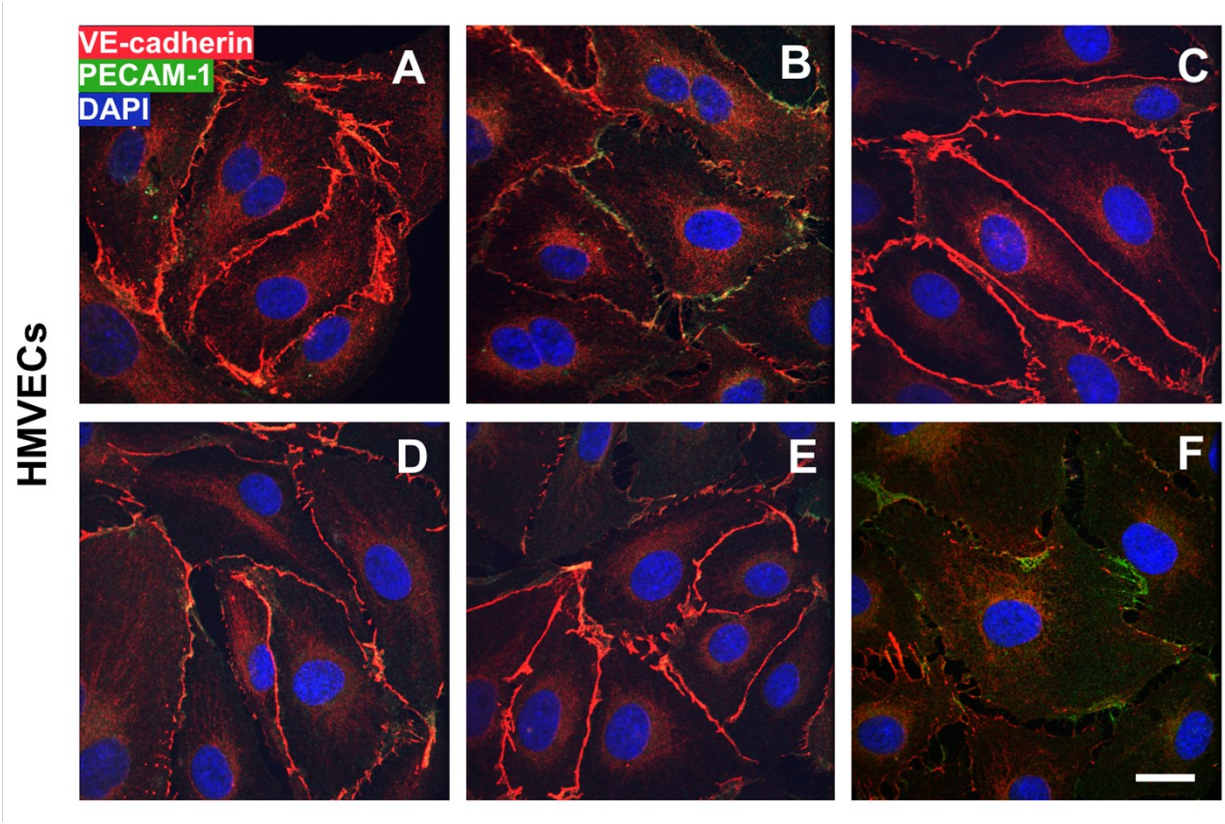
Supplementary Figure 7. The size of adherent (**a**) HUVECs and (**b**) HMVECs 24 h after cell seeding. Data is shown as change relative to F. n=3, no significant differences between the samples.



Supplementary Figure 8. Cell–fibronectin-interaction in HUVECs on surface A and C. Anti-FN immunofluorescence staining shows the interaction of HUVECs with the adsorbed FN, which is indicated by fibrillar structures (white arrow) and dark areas (white dotted arrow). Scale bars equal 100 µm.



Supplementary Figure 9. F-actin staining of HUVECs and HMVECs on the different fibronectin-coated surfaces. Scale bars equal 20 μm.



Supplementary Figure 10. VE-cadherin and PECAM-1 immunofluorescence staining of HMVECs on the different fibronectin-coated surfaces. Scale bars equal 20 µm.

Supplementary Table 1. C 1s core level peak fit parameters for sample B. Full width at half maximum values were established from the urethane peak at 289.6 eV and kept constant for all peaks (GP-FWHM = 1.206 eV; LP-FWHM = 0.418 eV; FWHM = 1.445 eV)

Peak name	Peak position, eV	rel. Area, %
C-O-C/C-OH	286.32	22.15
-C=O	287.70	4.38
-COOH	288.73	2.26

Supplementary Table 2. Mean values, standard deviations and statistical analysis of contact angles from uncoated and FN-coated samples.

	uncoated		FN-coated		uncoated versus FN-coated
Sample	**Mean**	***SD***	**Mean**	***SD***	**p-value***
A	17.5	*1.9*	45.0	*2.0*	$2.8*10^{-18}$
B	33.4	*2.7*	45.3	*2.0*	$1.3*10^{-9}$
C	48.2	*0.6*	57.2	*2.3*	$1.3*10^{-7}$
D	50.8	*1.7*	57.4	*1.5*	$5.2*10^{-6}$
E	54.3	*1.0*	58.7	*2.1*	0.00241
F	71.1	*1.6*	64.2	*1.1*	$1.0*10^{-5}$

* One-way ANOVA, n=3