

Supplementary Materials: A Micropatterning Strategy to Study Nuclear Mechanotransduction in Cells

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1. Calculation of Theoretical Contact Angle for a Monolayer Formation with HMDS

The Cassie equation [1] describes the expected contact angle of a solute on a bifunctional surface (in our case the piranha-treated coverslip glass surface and its HMDS layer) as follows:

$$\cos \Phi = f_1 \cos \theta_1 + f_2 \cos \theta_2, \quad (1)$$

where Φ is the equilibrium contact angle of water on the bifunctional surface (glass coverslip + HMDS), f_1 is the molecular fraction coverage of HMDS on the bifunctional surface, θ_1 is the expected contact angle of water on a pure HMDS surface (without coverslip support), f_2 is the molecular fraction of the bifunctional surface not covered by HMDS, and θ_2 is the expected contact angle of water on the pure glass coverslip surface (without HMDS).

f_1 and f_2 depend:

- (i) On the circular cross-sectional area taken up by the trimethylsilyl group ($\text{Si}-(\text{CH}_3)_3$) of HMDS, which has been estimated by Herzberg et al. [2] at $27.7 \text{ \AA}^2$

and

- (ii) On the number per surface area of hydroxyl silanol groups ($\text{Si}-\text{OH}$) that are available to react with HMDS on the coverslips. For a fully hydroxylated silica surface, this number has been estimated at 5 per $100 \text{ \AA}^2$ by different groups [3–5], and it is assumed to be unchanged for glass.

Thus, for a perfectly modified glass coverslip substrate where coverage and reaction of HMDS with silanol groups is optimal (monolayer), the surface molecular fraction coverage of trimethylsilyl should be $f_1 = (100/27.7)/5 = 0.722$. Consequently, we have a glass surface molecular fraction non-covered by HMDS of $f_2 = 1 - 0.722 = 0.278$.

The contact angle value of water on the piranha-treated glass coverslip surface (without HMDS) was measured and reported in Fernandez et al. [6] as $\theta_2 = 3.5^\circ$. There is, however, no known θ_1 value for a pure HMDS surface (without substrate support) because HMDS cannot polymerize on its own into a solid surface. However, the contact angle value of water on PDMS can be used as an appropriate estimate of θ_1 for HMDS because this polymer is extremely rich in methyl groups similar to those found in HMDS. Here, we used a contact angle of water on PDMS of 108° as determined by molecular simulation [7]. This value is in good agreement with reported experimental estimates between 98° and 112° (see references in Ismail et al. [7]). Thus, with values: $f_1 = 0.72$, $\theta_1 = 108^\circ$, $f_2 = 0.28$ and $\theta_2 = 3.5^\circ$ we can evaluate the expected equilibrium contact angle of water (Φ) for a perfect and monolayer coverage of HMDS on a glass coverslip, from Cassie's equation. We obtain $\cos \Phi = 0.054$ and $\Phi = 87^\circ$. This value is in excellent agreement with our measured value of $87 \pm 1^\circ$ after 90 min of reaction, thus indicating that vapor coating provide a homogenous monolayer deposition of HMDS on glass coverslips.

We note that multilayer polymerization of HMDS at the coverslip surface (although it is improbable considering the nature of the chemical reaction) would result in water contact angles that would increase toward 108° overtime. Similarly, sub-monolayer coverage would result in water contact angles that would decrease from the theoretical 87° value towards the 3.5° angle value measured for piranha-treated glass.

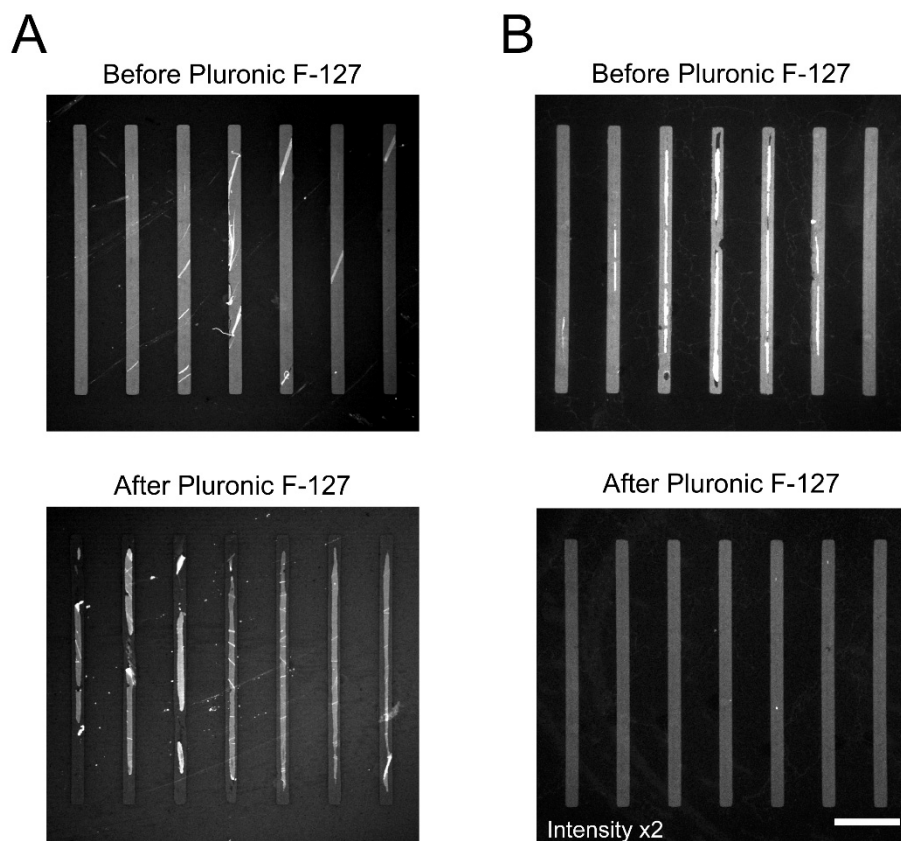


Figure S1. Effect of PF-127 treatment on fibronectin attachment to APTES- and GPTMS-coated coverslips. (A) fluorescence confocal images of fibronectin microstamped from $210 \times 10 \mu\text{m}$ rectangular PDMS stamps on APTES-coated coverslips before and after treatment with PF-127; (B) fluorescence confocal images of fibronectin microstamped from $210 \times 10 \mu\text{m}$ rectangular PDMS stamps on GPTMS-coated coverslips before and after treatment with PF-127. Note that the fluorescence intensity in the microscopy image after pluronic treatment for GPTMS was multiplied by two to facilitate a visualization of the micropatterns. Scale bar for all images: $50 \mu\text{m}$.

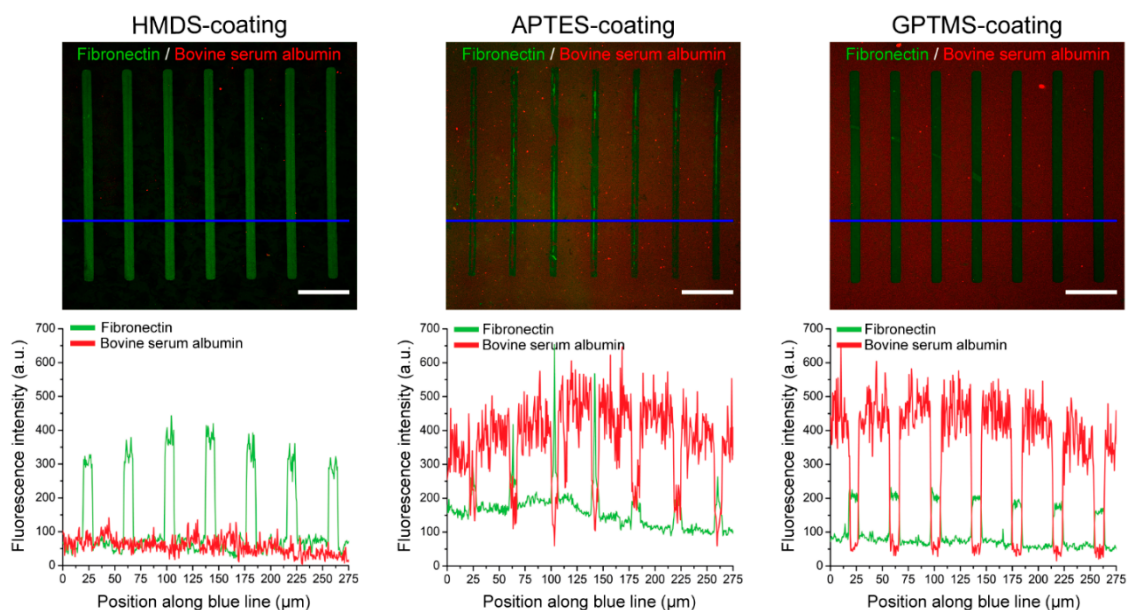


Figure S2. Antifouling efficacy of PF-127 treatment on HMDS-, APTES- and GPTMS-coated coverslips after fibronectin attachment. **Top row:** Dual-color fluorescence confocal images of fibronectin microstamped from $210 \times 10 \mu\text{m}$ rectangular PDMS stamps (green) and of A647-BSA non-specifically

binding outside micropatterns (red) on HMDS-, APTES- and GPTMS-coated coverslips after treatment with PF-127. All scale bars: 50 μm . **Bottom row:** Fluorescence intensity profiles for fluorescently labeled fibronectin (green) and A647-BSA (red) along the blue line in each of the corresponding confocal images from the top row.

2. Measurements of Contrast Values to Compare the Quality of Fibronectin Microcontact Printing across Coverslips Coated with Different Organosilanes

To quantify the quality of the fibronectin micropatterns, fluorescence intensities of Cy3B-fibronectin along microcontact printed strips ($n = 15\text{--}25$ rectangular $10 \times 210 \mu\text{m}$ strips) on multiple coverslips were measured for the three organosilane coatings, before and after PF-127 treatments. Specifically, the mean contrast fluorescence values $\pm$ standard deviation were calculated as previously described [8] as:

$$C = \frac{I_{\text{on}} - I_{\text{off}}}{I_{\text{on}}}, \quad (2)$$

where C is the mean contrast value, I_{on} is the mean fluorescence intensity within the rectangular fibronectin strips where fibronectin transfer occurs, and I_{off} is the mean fluorescence intensity in-between strips, where no transfer should occur. These results are reported in Supporting Table S1.

Table S1. Quality assessment of fibronectin micropatterns.

Coating	Contrast Value $\pm$ Standard Deviation	
	Before Pluronic F127	After Pluronic F127
HMDS	0.71 ± 0.04	0.67 ± 0.07
APTES	0.57 ± 0.10	0.28 ± 0.11
GPTMS	0.79 ± 0.11	0.72 ± 0.06

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