

## Quasi-3D morphology and modulation of focal adhesions of human adult stem cells through combinatorial concave elastomeric surfaces with varied stiffness

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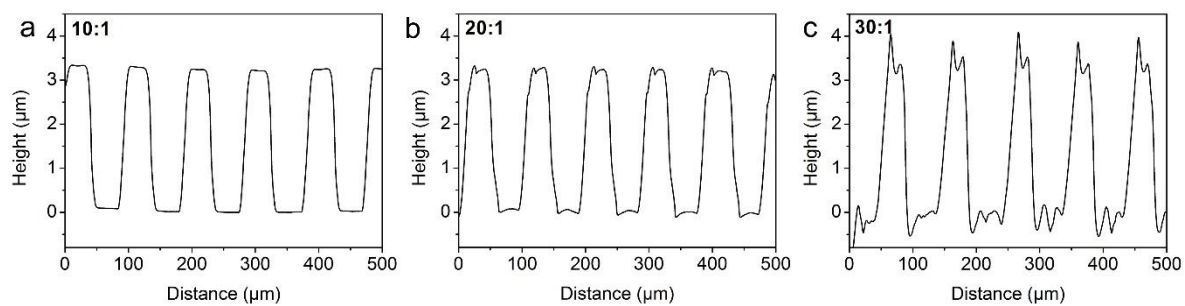
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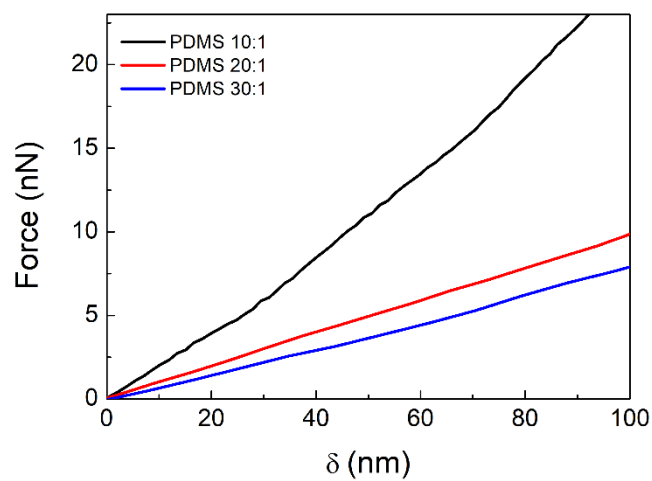
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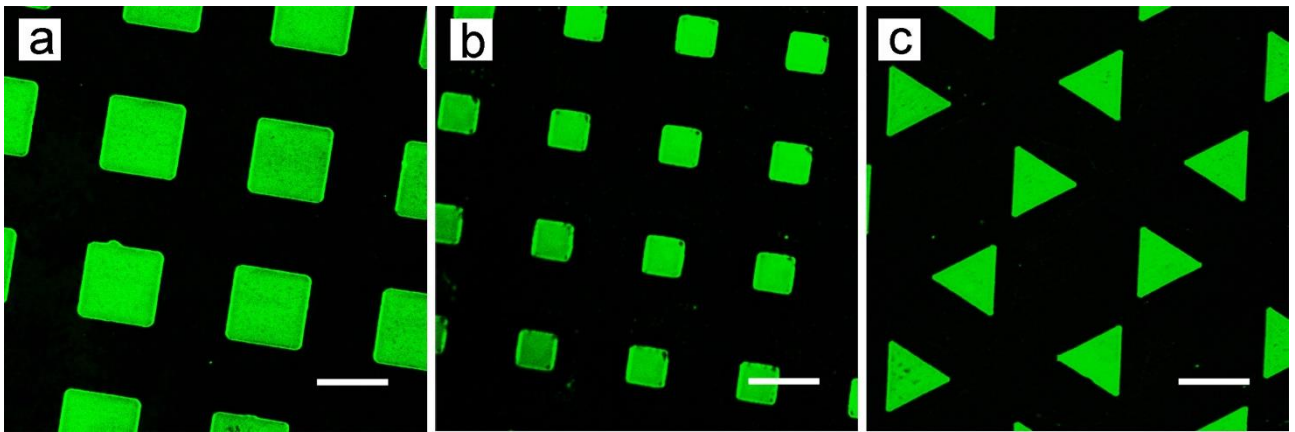
### ELECTRONIC SUPPLEMENTARY INFORMATION



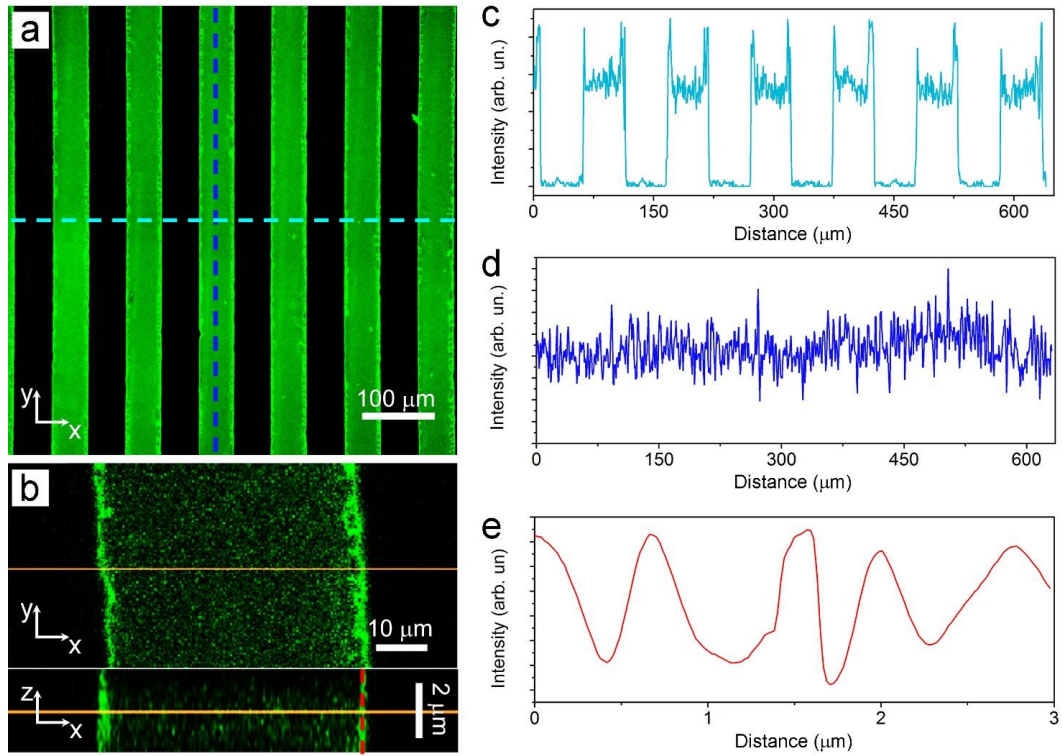
**Fig. S1.** Morphological profiles of 10:1 (a), 20:1 (b) and 30:1 (c) microstructured PDMS surfaces. The 10:1 profile (a) more closely represents the actual sample morphology, being free from distortions affecting softer surfaces.



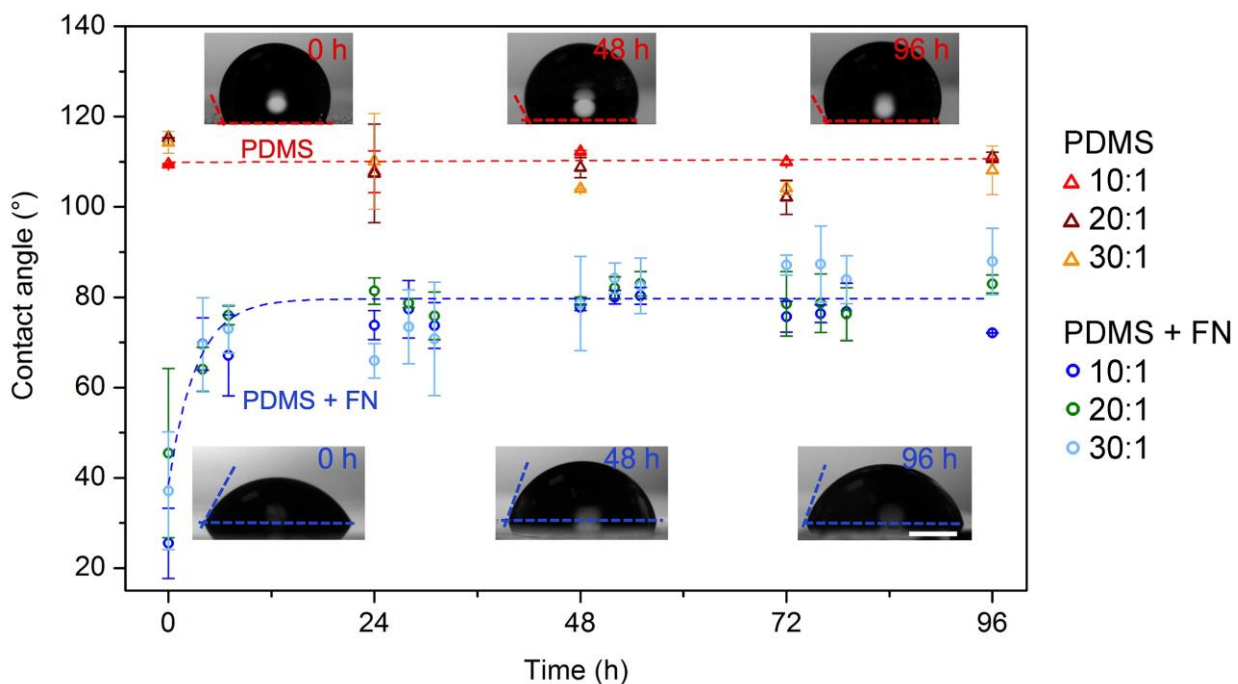
**Fig. S2.** Force-indentation curves for PDMS surfaces with different prepolymer base to curing agent ratio. A constant is added to the indentation to make it zero at the point of contact.



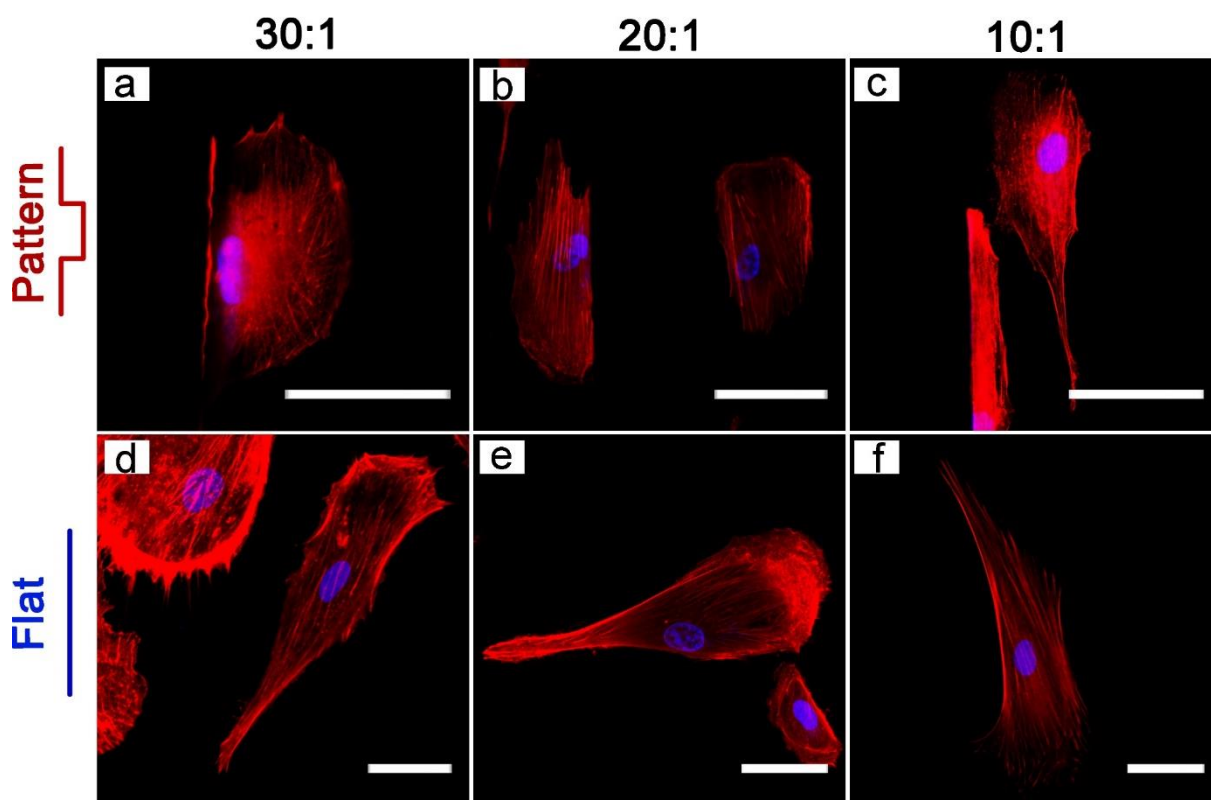
**Fig. S3.** Microstructured PDMS surfaces with FN-functionalized microwells, presenting different patterns: 50  $\mu\text{m}$ - and 25  $\mu\text{m}$ -side squares (a and b, respectively), and 50  $\mu\text{m}$ -side triangles (c). FN is highlighted by FITC staining (green). The black background is indicative of protein removal from the top of the PDMS microstructures following printing. Scale bars = 50  $\mu\text{m}$ .



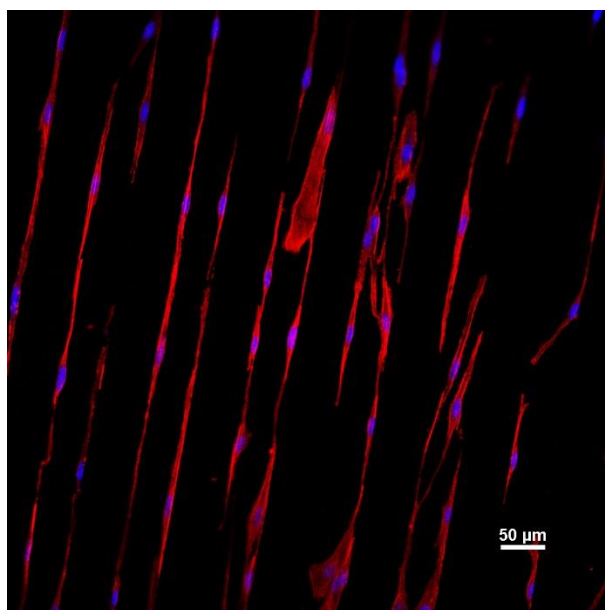
**Fig. S4.** (a,b) Confocal micrographs of PDMS grooves, functionalized with FN. FN is highlighted by FITC staining (green). The bottom image in (b) shows a confocal Z-stack micrograph of immunolabeled FN in the groove. (c-e) Intensity profiles of immunostained FN along the light blue dashed line in (a) (x direction, c), the dark blue dashed line in (a) (y direction, d) and the red dashed line in (b) (z direction, e). In all the directions, the intensity from immunostained FN does not highlight presence of protein gradients.



**Fig. S5.** WCA values for FN-functionalized, 10:1, 20:1 and 30:1 PDMS flat surfaces (circles) and for untreated PDMS (triangles). Insets: optical micrographs of water droplets on 1:10 substrates after 0, 2 and 4 days after functionalization. The dashed lines are guides for the eyes. Scale bar = 1 mm.

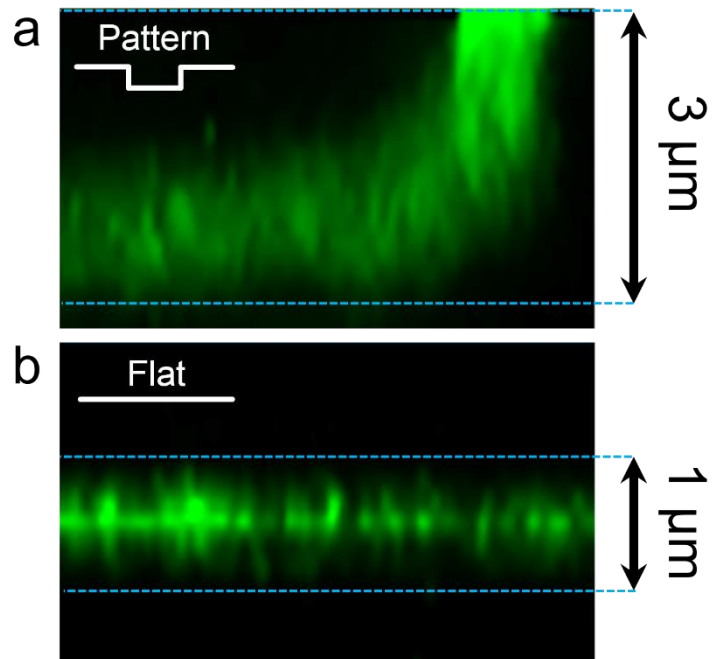


**Fig. S6.** Confocal imaging of isolated cells. The mean area of isolated cells cultured on patterned substrates (a-c) is in the range  $2-3 \times 10^3 \mu\text{m}^2$ , whereas the mean area of cells on flat surfaces is in the range  $8-9 \times 10^3 \mu\text{m}^2$ . Mean nuclear area:  $130-170 \mu\text{m}^2$  for cells on patterned surfaces and  $150-250 \mu\text{m}^2$  for cells on flat surfaces, respectively.



**Fig. S7.** ARPCs on a patterned, 10:1 PDMS substrate. Cells show highly preferential interaction with the groove edges (e.g., base-wall corners), and they do not cover the central regions of the patterned grooves.





**Fig. S8.** Zoom of differently configured cell membranes by vinculin staining, for patterned (a) and flat (b) surfaces. Imaged regions are highlighted by red squares in Fig. 7a and 7e, respectively.