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Supporting Material

Cell Shape-Regulation of Smooth Muscle Cell Proliferation

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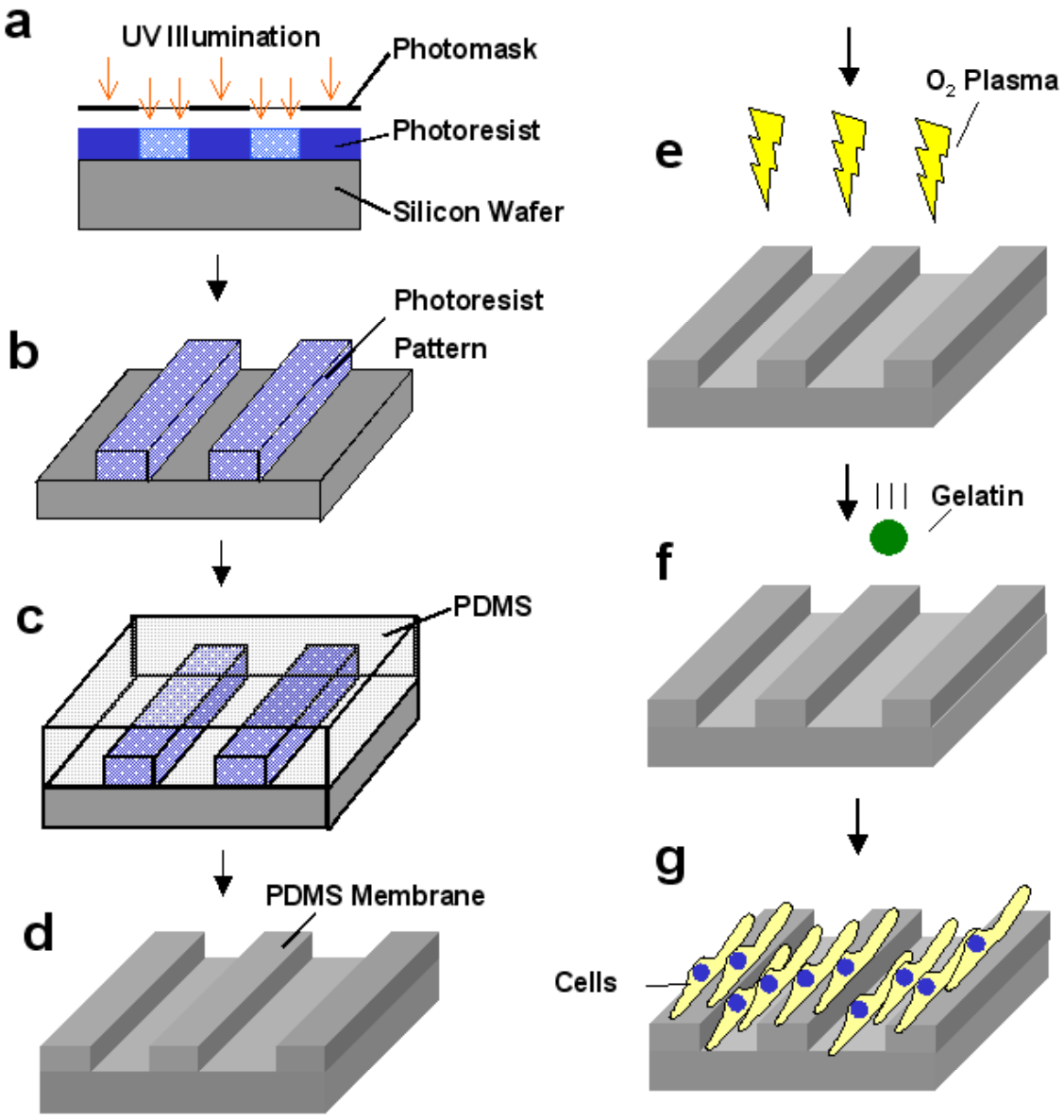
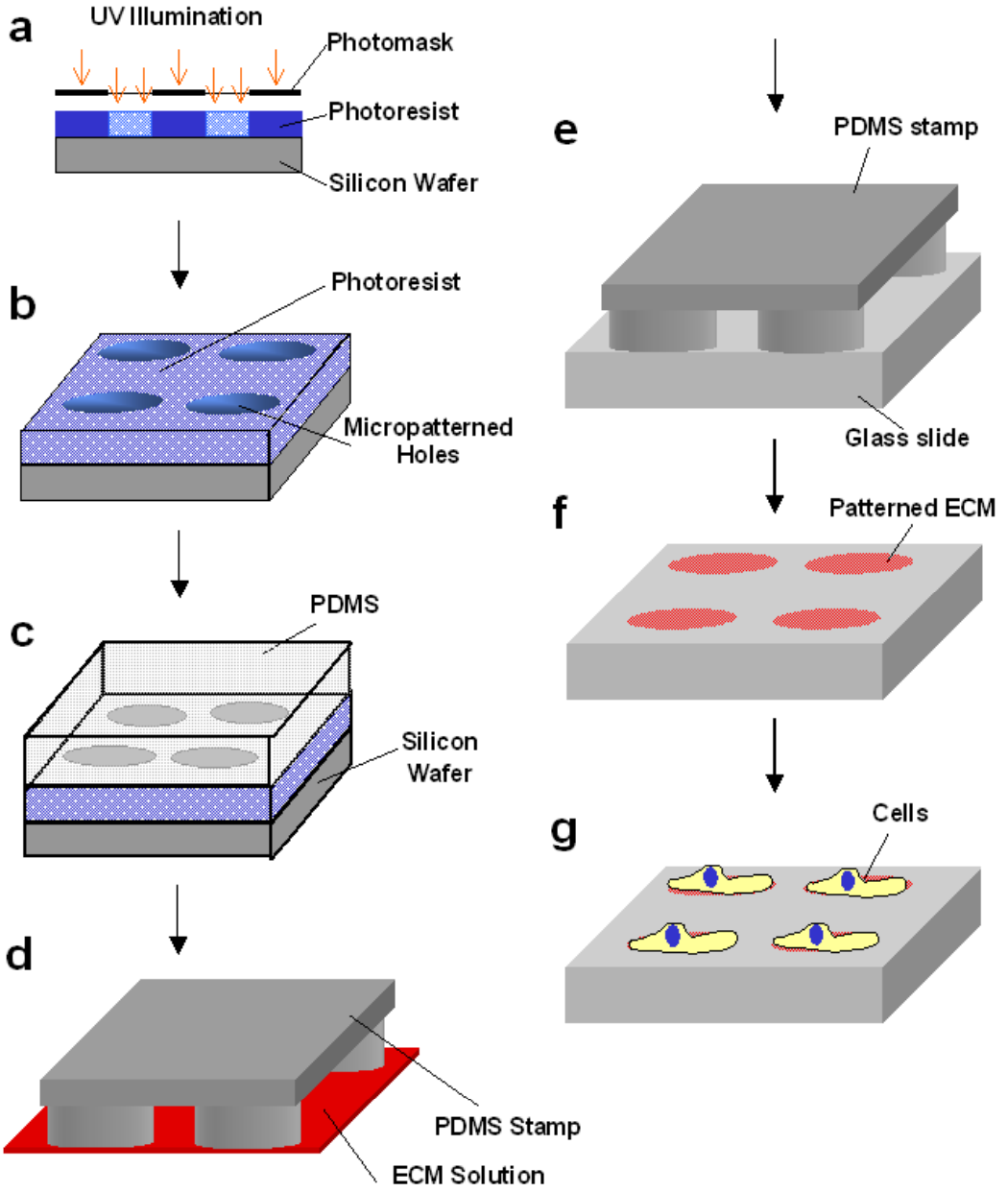
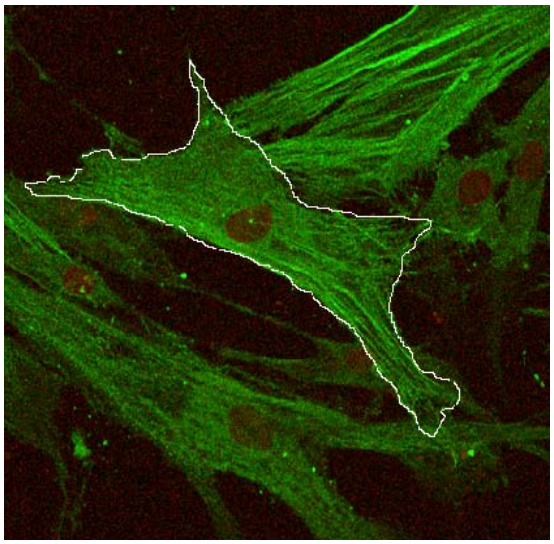


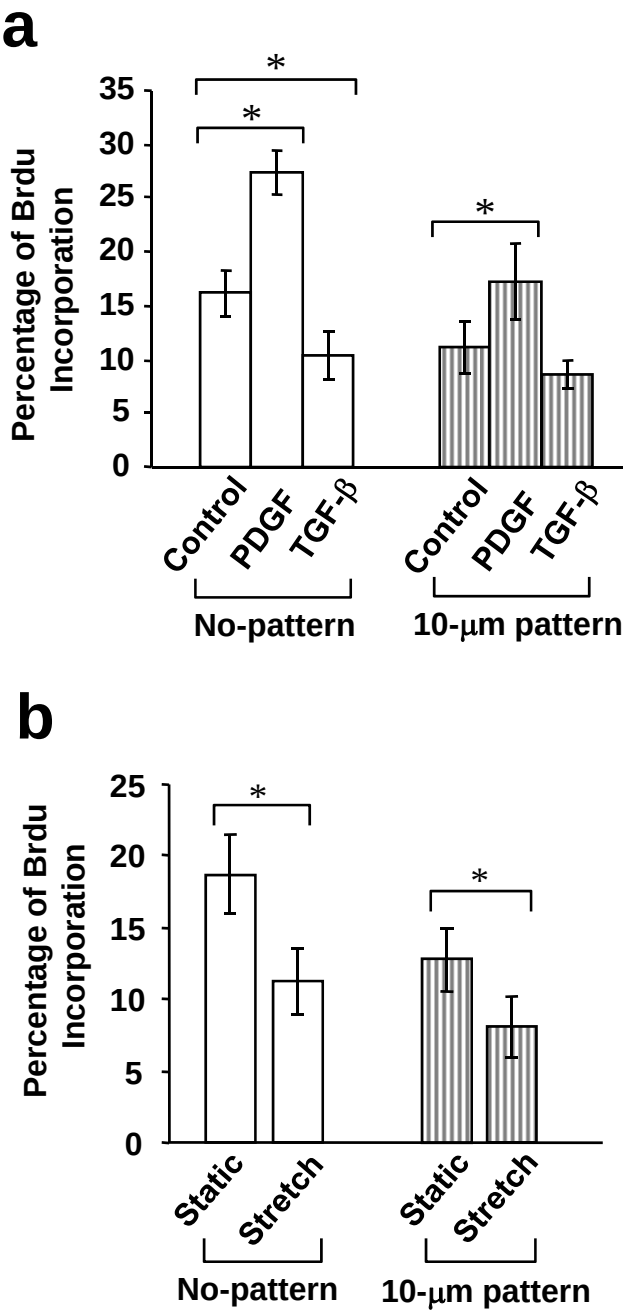
Supplementary Figure 1. Procedure for micropatterning topographies and cells. **a.** Positive photoresist was spin-coated on silicon wafer, baked at 90°C for 60 seconds, and exposed to UV light for 11 seconds (at UV Lamp Intensity of 18W/KS) through a photomask with parallel 10-μm strips. **b.** After doing a post-bake in 120°C oven for 20 seconds, the wafers were developed in OPD 4262, thus leaving a patterned surface. **c.** PDMS was poured onto the silicon master molds, and spun at 185 RPM for 2 min to ensure that all PDMS membranes would be 250-mm thick. **d.** After the PDMS cured, the micropatterned membranes were peeled from the silicon masters molds. Non-pattern PDMS membranes were made in the same manner, except their silicon master molds were blank silicon wafers. The PDMS mold was cleaned in ultrasonic water bath. **e.** PDMS mold was exposed to O_2 plasma to increase its hydrophilicity. **f.** 2% gelatin was used to coat the surface to aid cell adhesion. **g.** SMCs were seeded.



Supplementary Figure 2. The microstamping procedure used to create areas of defined adhesion islands. **a.** Positive photoresist was spin-coated on silicon wafer and exposed to UV light through a photomask. **b.** Photoresist was developed thus leaving a patterned surface. **c.** PDMS was cast onto the silicon wafer and cured. **d.** The PDMS stamp was peeled from the silicon mold, washed, cleaned, and dried. An ECM solution was then adsorbed onto microtextured side of the PDMS stamp. **e.** PDMS stamps were incubated with fibronectin solution (0.33 mg/mL), then dried, washed, and placed in conformal contact with di-amino-PEG-sulfo-SANPAH slides. **f.** The PDMS stamp was removed after 30 min and adsorbed fibronectin was crosslinked to the slides by activating the sulfo-SANPAH molecules under UV light for 5 min. The slides were then incubated in phosphate buffered saline (PBS) for 15 minutes at room temperature. Finally, the slides were incubated in 10% glycine (Sigma) in PBS solution (pH 7.4) for 30 minutes to quench active sulfo-SANPAH molecules. **g.** SMCs were seeded on the slides and unbound cells were washed off after 30 – 60 min based on visual observations. One cell per microstamped adhesion island was achieved by titrating the seeding density of cells.



Supplementary Figure 3. An example showing the outline of cell boundary for shape analysis. Cells were immunostained for α-actin, and the outline of cells was used to quantify cell morphology.



Supplementary Figure 4. Effects of growth factors and cyclic uniaxial mechanical strain on SMC proliferation on no-pattern and micropatterned surfaces. **a**. Effects of PDGF and TGF-β on SMC proliferation. SMCs were cultured on the no-pattern or micropatterned PDMS for 24 hr, and PDGF (10 ng/ml) or TGF-β (10 ng/ml) was added to the medium for another 24 hr culture. Then SMCs were incubated with BrdU for 2 hr. The cells were fixed and doubled stained for BrdU and nuclei. The percentage of BrdU-positive cells (the cells in S phase) was calculated (>200 cells per group). **b**. Effects of cyclic uniaxial mechanical strain on SMC proliferation. SMCs cultured on the no-pattern or micropatterned PDMS were subjected to cyclic (1 Hz) uniaxial mechanical strain (5%) for 24 hr, and the cells were subjected to proliferation analysis as in **a**. In both **a** and **b**, bars represent means ± SD. Data were extracted from at least three experiments. *Statistical significance (at least $P < 0.05$).

Supplementary Table 1.

Genes with more than 2-fold changes (SMCs on 10-μm microgrooves / SMCs on non-pattern surfaces)

UniGene Name	UniGene Symbol	LocusLink ID	Average of fold change
nuclear receptor subfamily 4, group A, member 3	NR4A3 (NOR-1)	8013	0.289
heat shock 70kDa protein 6 (HSP70B)	HSPA6	3310	0.291
chemokine orphan receptor 1	CMKOR1	57007	0.443
nuclear receptor subfamily 4, group A, member 2	NR4A2	4929	0.448
radical S-adenosyl methionine domain containing 2	RSAD2	91543	0.485
chromosome 16 open reading frame 34	C16orf34	90861	2.055
pro-melanin-concentrating hormone	PMCH	5367	2.135