Electronic Supplementary Material (ESI) for Biomaterials Science.

Supplementary Information

Combined effects of multi-scale topographical cues on stable cell sheet formation and differentiation of mesenchymal stem cells

Sisi Li†[a], Shreyas Kuddannaya†[a], Yon Jin Chuah[b], Jingnan Bao[a], Yilei Zhang[a], Dongan Wang[b]*

[a]School of Mechanical and Aerospace Engineering, Nanyang Technological University, 50 Nanyang Avenue, Singapore 639798, Singapore.

[b]School of Chemical and Biomedical Engineering, Nanyang Technological University, 62 Nanyang Drive, Singapore 637459, Singapore

**E-mail: ylzhang@ntu.edu.sg*

†These authors contributed equally to this work.

1. Biomarker information

Table S1: Primer sequences for specific genes in real time polymerase chain reaction

Gene	Forward primer sequence (5'-3')	Reverse primer sequence (5'-3')
Collagen type-1 (Col1)	CAGAACGGCCTCAGGTACCA	CAGATCACGTCATCGCACAAC
Alkaline phosphatase (ALP)	ATGAGCTCAACCGGAACAA	GTGCCCATGGTCAATCCT
Osteocalcin (OCN)	TCAACCCCGACTGCGACGAG	TTGGAGCAGCTGGGATGATGG
RUNX2	GAGGAACCGTTTCAGCTTACTG	CGTTAACCAATGGCACGAG
GAPDH	GCTTTGCCCCGCGATCTAATGTTC	GCCAAATCCGTTCACTCCGACCTT

2. Cytoskeletal profiles and coverage of adherent cell layers (low cell density)

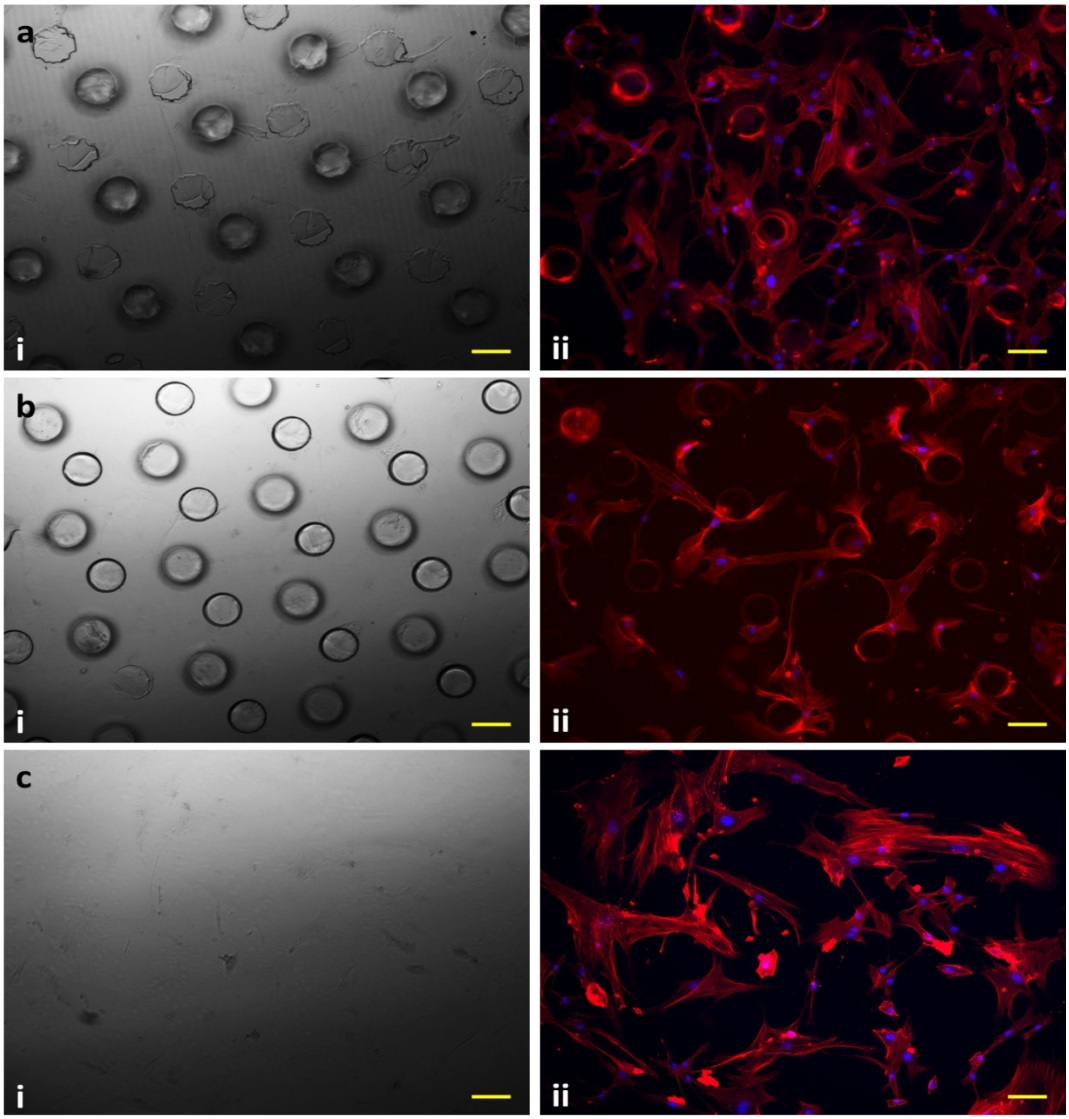

Fig. S1: Phase contrast and fluorescent images of cytoskeletal and nucleus stained hMSCs in low cell density on (a) H-P-G (b) H-P and (c) Flat-PDMS substrates. Scale bar: 100 µm.

3. hMSC morphology (SEM) on H-P-G and H-P substrates

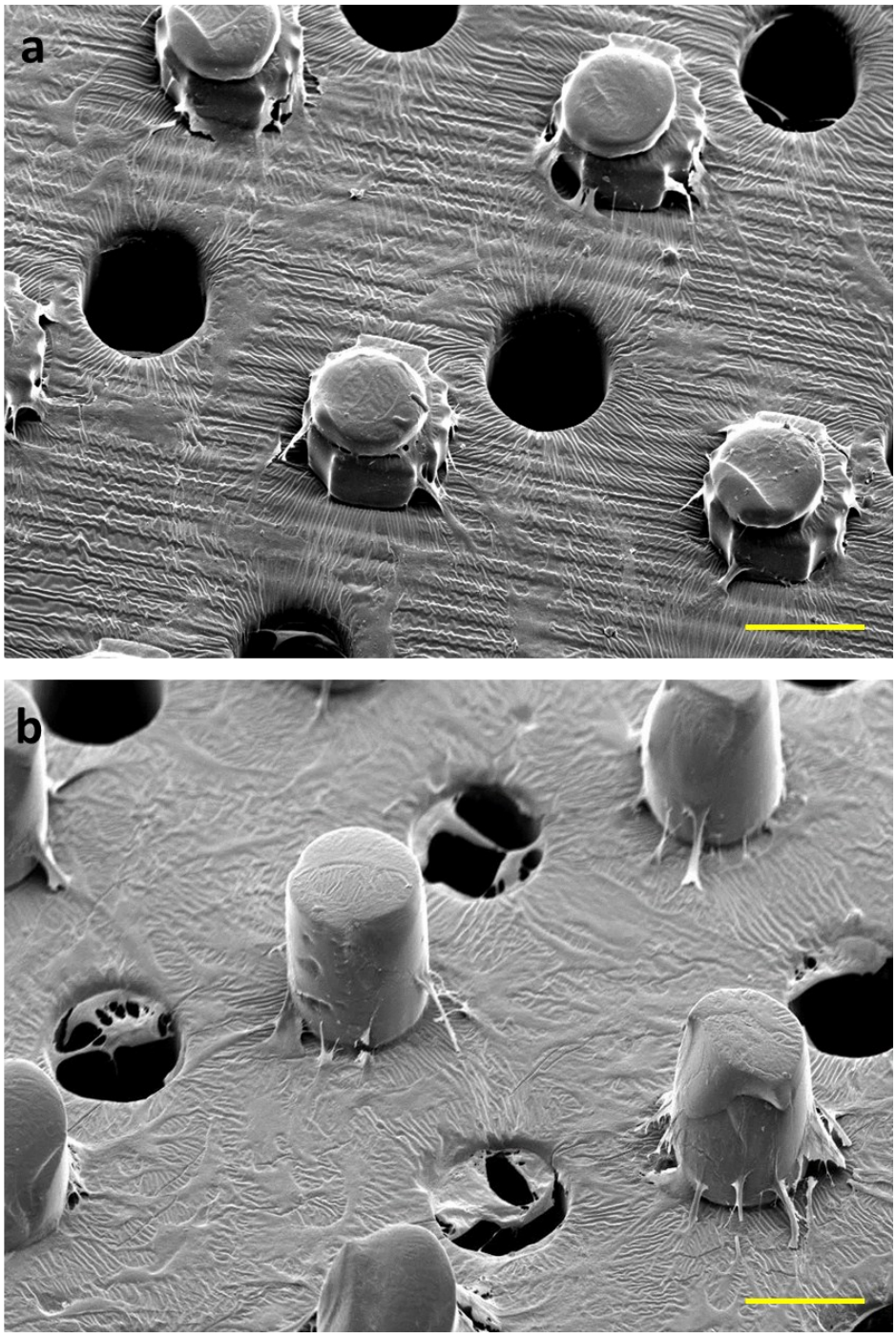

Fig. S2: Enlarged SEM micrograph revealing detailed morphology of hMSCs around pillar and hole structures on the hole, pillar and groove based (a) H-P-G and (b) H-P substrates. Random cell orientations on the flat regions of the substrates is also seen in H-P substrate. Scale bar: 100 µm

4. Cell morphology and alignments in holes

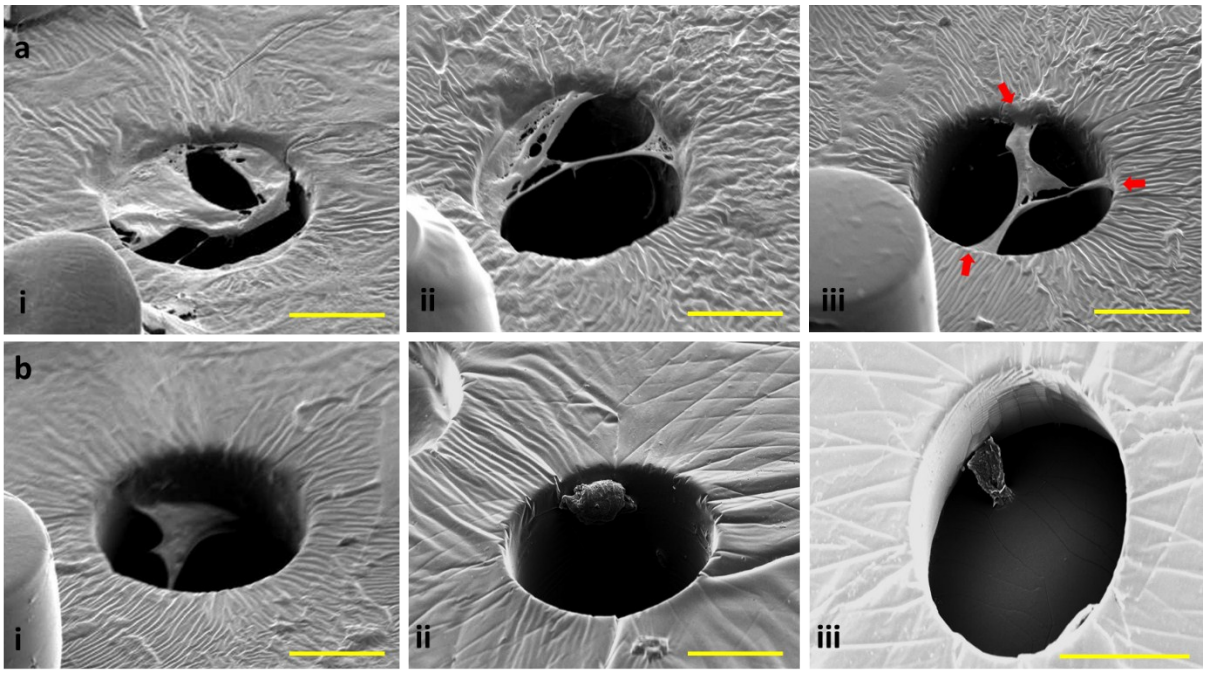

Fig. S3: (a) Morphology of hMSCs covering the surface of the holes as (i) wide cell sheet (ii) partial cell sheet and (iii) stretched single cell. (b) hMSC migration and attachment inside the hole as (i) sinking cell sheet (ii) single cell attached in the inner wall (iii) deeply migrated single cell attahced between the inner wall and the supporting basal surface. Scale bar: 50 µm.

5. Microfabrication of multi-topographic thin films and application as rolled scaffold

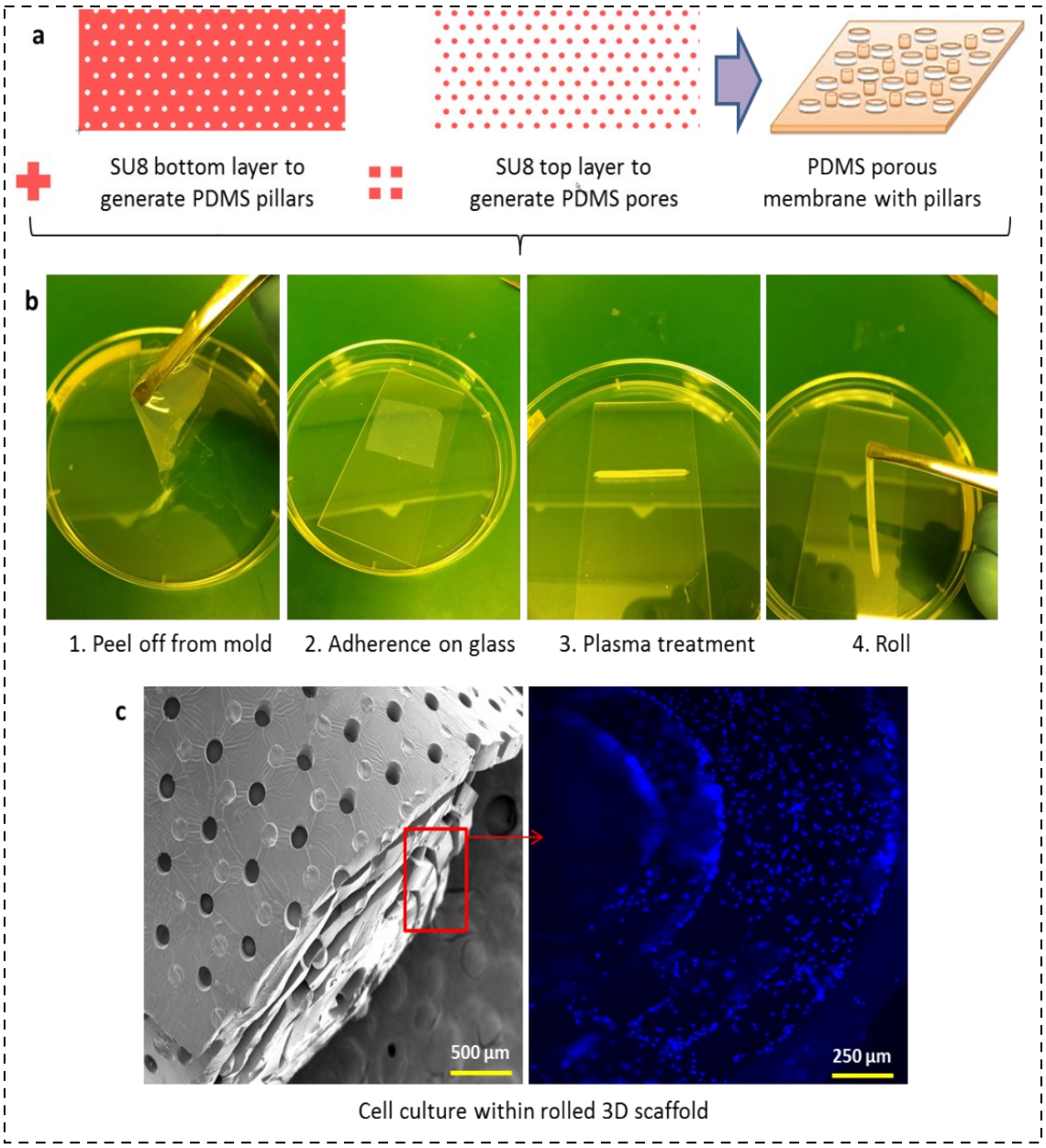

Fig. S4: (a) Steps involved in negative photoresist (SU-8) layer by layer alignment and developing to obtain a stencil/membrane like hole+pillar feature on the thin (100µm thick spin-coated and cured) PDMS. (b) Steps involved in preparing a 3D roll from the thin micro-fabricated PDMS (c) (Left) SEM micrograph of the rolled PDMS. The spaces between the adjacent layers is due to the spacing of 100 µm which is the height of the pillars which create the spaces upon rolling (Right) cell infiltration into the scaffold demonstrated by DAPI (nuclear) staining.