Culture substrate made of elastomeric micro-tripod arrays for long-term expansion of human pluripotent stem cells

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Contact angle measurement. The sessile drop method was used to measure the contact angle of a water droplet on the micro-tripod arrays (MTAs) and flat PDMS using a microscope and a CCD camera. A 2 µl water droplet was deposited onto the sample surface and images were taken water with the CCD camera. The edge of the droplet was then analysed using a sessile drop-fitting model.

Circulation underneath the contact area. To prove the circulation of culture medium underneath the contact area of the hPSC colonies, a glass capillary of 1 mm diameter was brought into conformal contact with a MTAs and a flat PDMS layer, respectively. 1 mL of culture medium was then deposited in the area around the capillaries. 5 min later, after reaching a stabilized capillary length, the culture medium outside the capillaries was removed and a photograph was taken, as shown in Figure S3. Clearly, the culture medium could be circulating underneath the contact area of the capillary with the MTAs but not the flat PDMS layer, resulting in the observed capillary effect. In the case of free entrance, the capillary length of liquid is given by $h = 2\gamma cos\vartheta / \rho g R$, where γ is the surface tension and ρ is the density of the liquid, g is the acceleration due to gravity, R is the radius of the capillary and ϑ is the contact angle of the liquid. By using γ, ρ and ϑ parameters of water, a capillary length of

29.3 mm could be obtained. In practice, due to the loss of the medium after removal of outside liquid, we only measured a capillary length of about 10.98 ± 2.75 mm from the photograph.

Primers	Sequences
OCT4 forward	5′ -GACAGGGGGAGGGGAGGAGCTAGG-3′
OCT4 reverse	5′ -CTTCCCTCCAACCAGTTGCCCCAAAC-3′
NANOG forward	5′ -CAGCCCCGATTCTTCCACCAGTCCC-3′
NANOG reverse	5′ -CGGAAGATTCCCAGTCGGGTTCACC-3′
SOX2 forward	5′ -GGGAAATGGGAGGGGTGCAAAAGAGG-3′
SOX2 reverse	5′ -TTGCGTGAGTGTGGATGGGATTGGTG-3′
SOX1 forward	5′ -GCGGAAAGCGTTTTCTTTG-3'
SOX1 reverse	5′ - TAATCTGACTTCTCCTCCC-3′
BRACHYURY forward	5′ -GCCCTCTCCCTCCCCTCCACGCACAG-3′
BRACHYURY reverse	5′ -CGGCGCCGTTGCTCACAGACCACAGG-3′
AFP forward	5′ -GAATGCTGCAAACTGACCACGCTGGAAC-3′
AFP reverse	5′ -TGGCATTCAAGAGGGTTTTCAGTCTGGA-3′
GAPDH forward	5′ - ACCACAGTCCATGCCATCAC-3′
GAPDH reverse	5′ - TCCACCACCCTGTTGCTGTA-3′

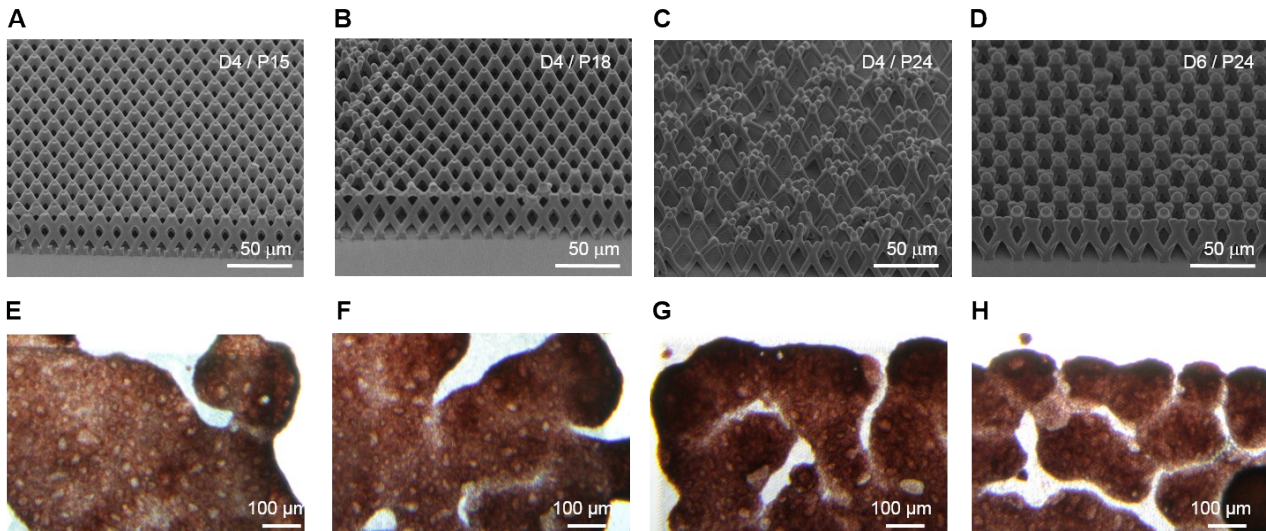**Table S1.** Primer sets for pluripotency markers

Figure S1. MTAs obtained by casting with a resist template that was produced by backside photolithography through a Cr mask of holes of different diameter (D) and pitch size (P). (A) D = 4 µm and P = 15 µm. (B) D = 4 µm and P = 18 µm. (C) D = 4 µm and P = 24 µm. (D) D = 6 µm and P = 24 µm. hiPSCs were cultured on substrates with different pillar diameter and pitch size for 4 days, and AP staining was performed as shown in (E), (F), (G), and (H) respectively.
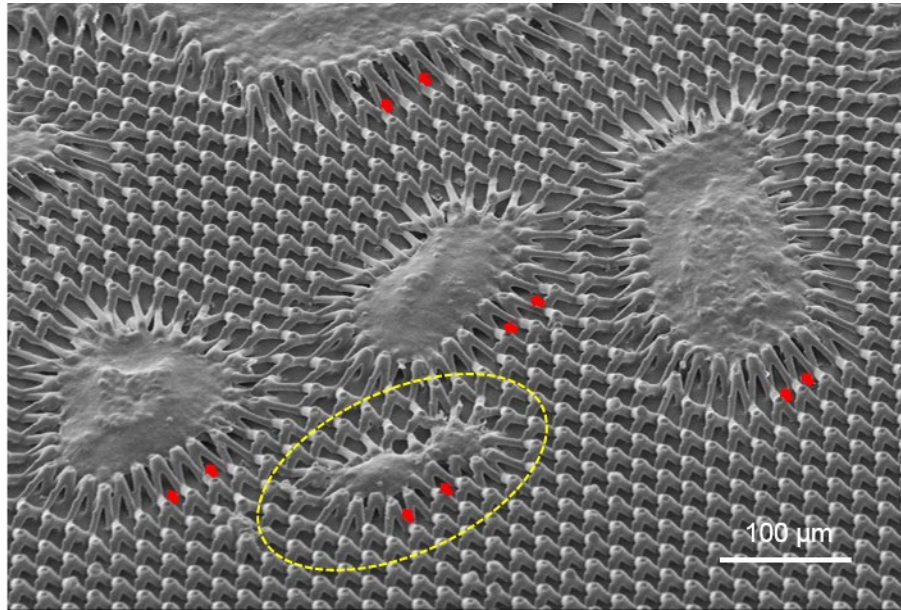
Figure S2. Adhesion of hiPSCs on MTA. hiPSC colonies on gelatin-coated MTAs were observed at day 3 of passage 1 (P1) by scanning electron microscopy. The dotted circle marks a small colony composed of several cells that dramatically deformed the surrounding pillars. The space between the colony body and the substrate is marked by red arrows.
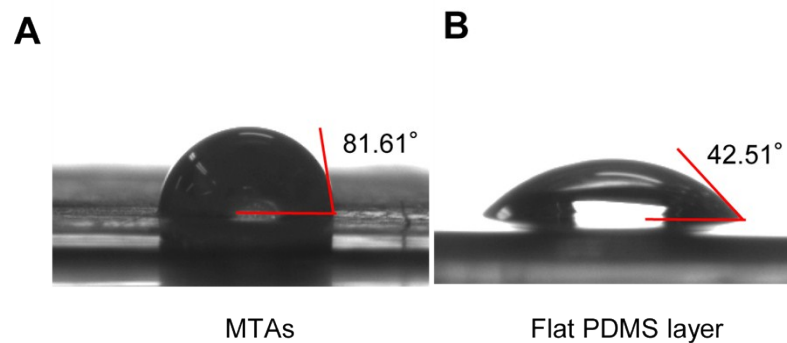

Figure S3. Photographs of water droplet on a gelatin coated MTAs (A) and a flat PDMS layer (B), indicating different wettability. The sessile drip method was used to measure the contact angle of a water droplet on the micro-tripod arrays (MTAs) and flat PDMS layer using a microscope and a CCD camera. A 2 µl water droplet was deposited onto the sample surface and the water substrate interface was imaged with the CCD camera. The edge of the droplet was then deduced using a sessile drop-fitting model.
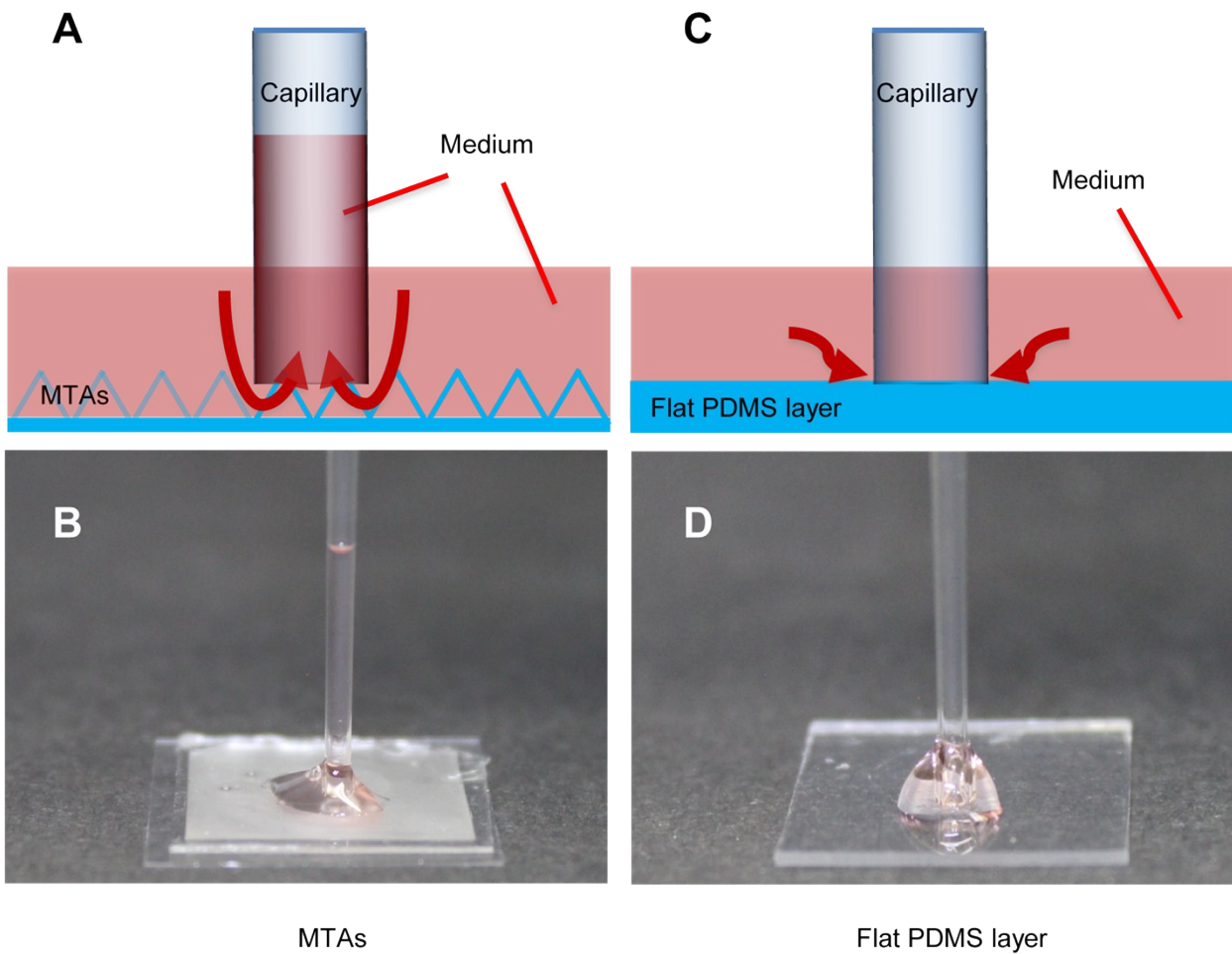
Figure S4. Diagrams and Photographs of a 1 mm diameter capillary placed on the surface of MTAs (A, B) and a flat PDMS layer (C, D). Due to conformal contact, liquid (culture medium) deposited outside the contact area of the flat PDMS layer cannot go into the capillary whereas it partially fits the capillary when deposited outside the contact area of the MTAs due to underneath circulation and capillary effect. The photos were taken after the liquid level inside the capillary became stable.
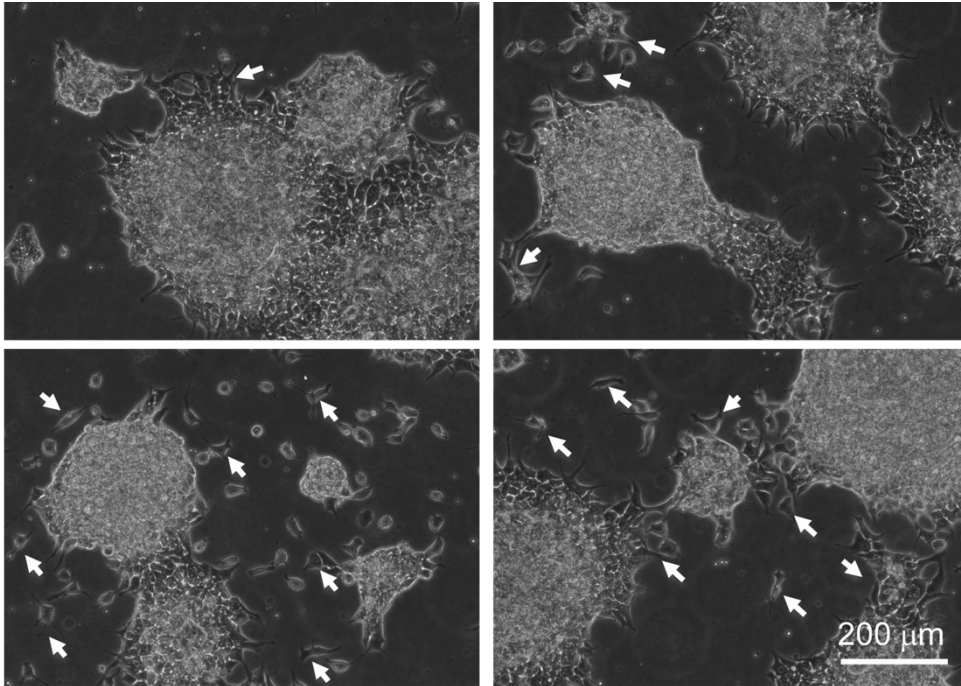
Figure S5. Photographs of hiPSCs on gelatin-coated flat PDMS (passage 7), showing spontaneous differentiation (marked by white arrows) in the edge area of the colonies.
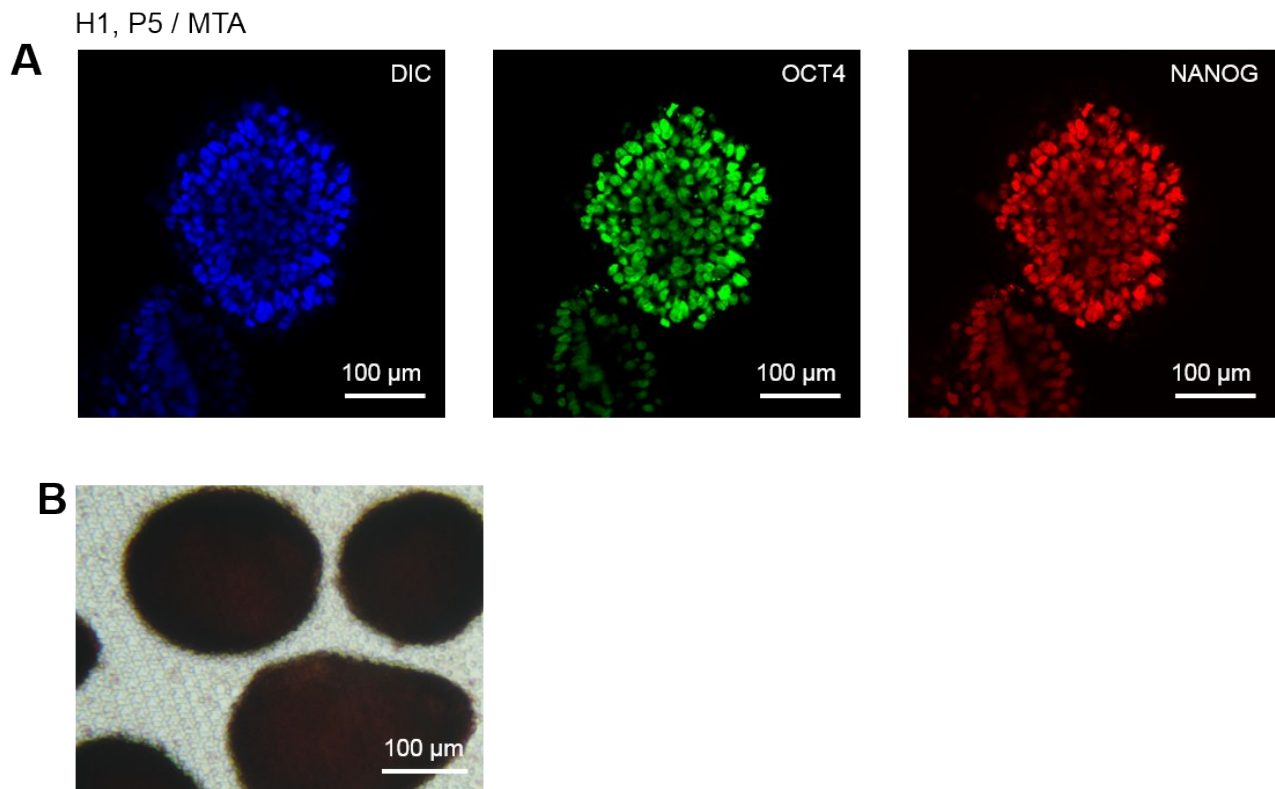
H1, P5 / MTA

A

B

Figure S6. Immunofluorescence (A) and AP staining (B) images of hESC (H1) colonies on gelatin-coated MTAs at passage 5 (P5).
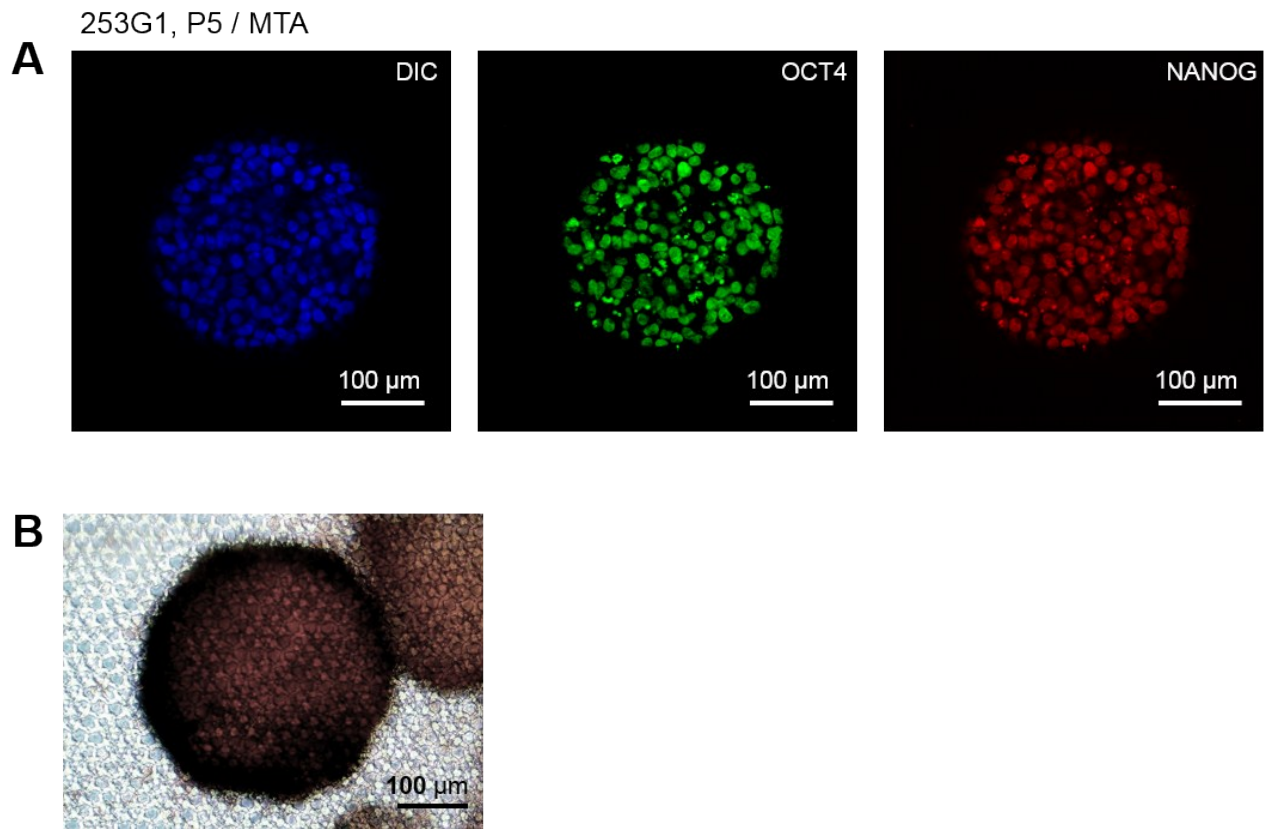
Figure S7. Immunofluorescence (A) and AP staining (B) images of hiPSC (253G1) colonies on vitronectin-coated MTAs with E8 culture medium at passage 5 (P5).