Supporting Information

Synthetic extracellular matrices with nonlinear elasticity regulate cellular organization

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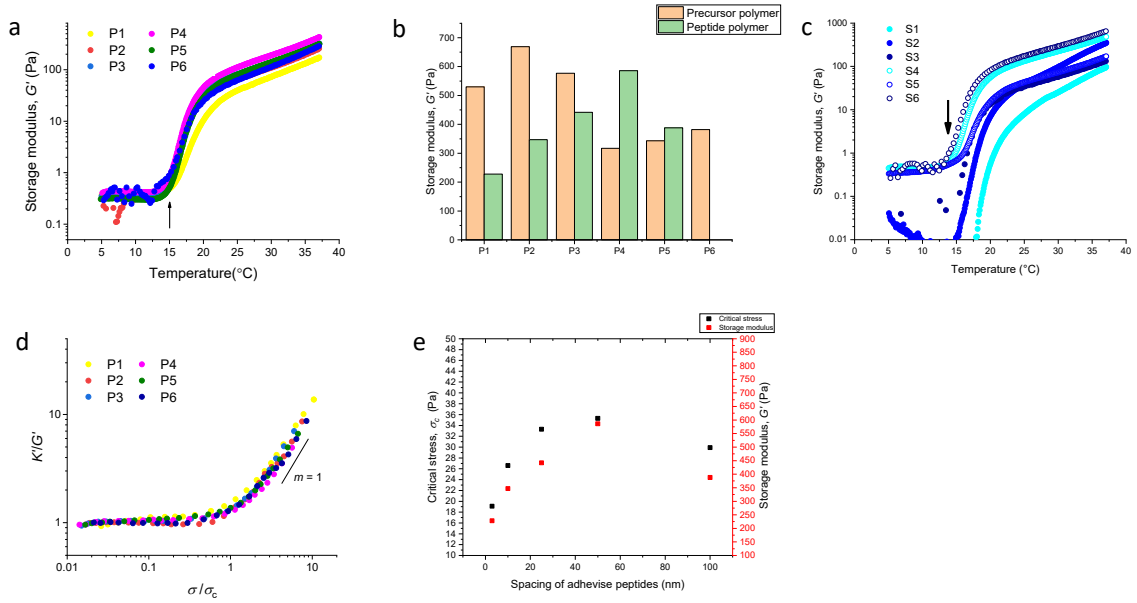
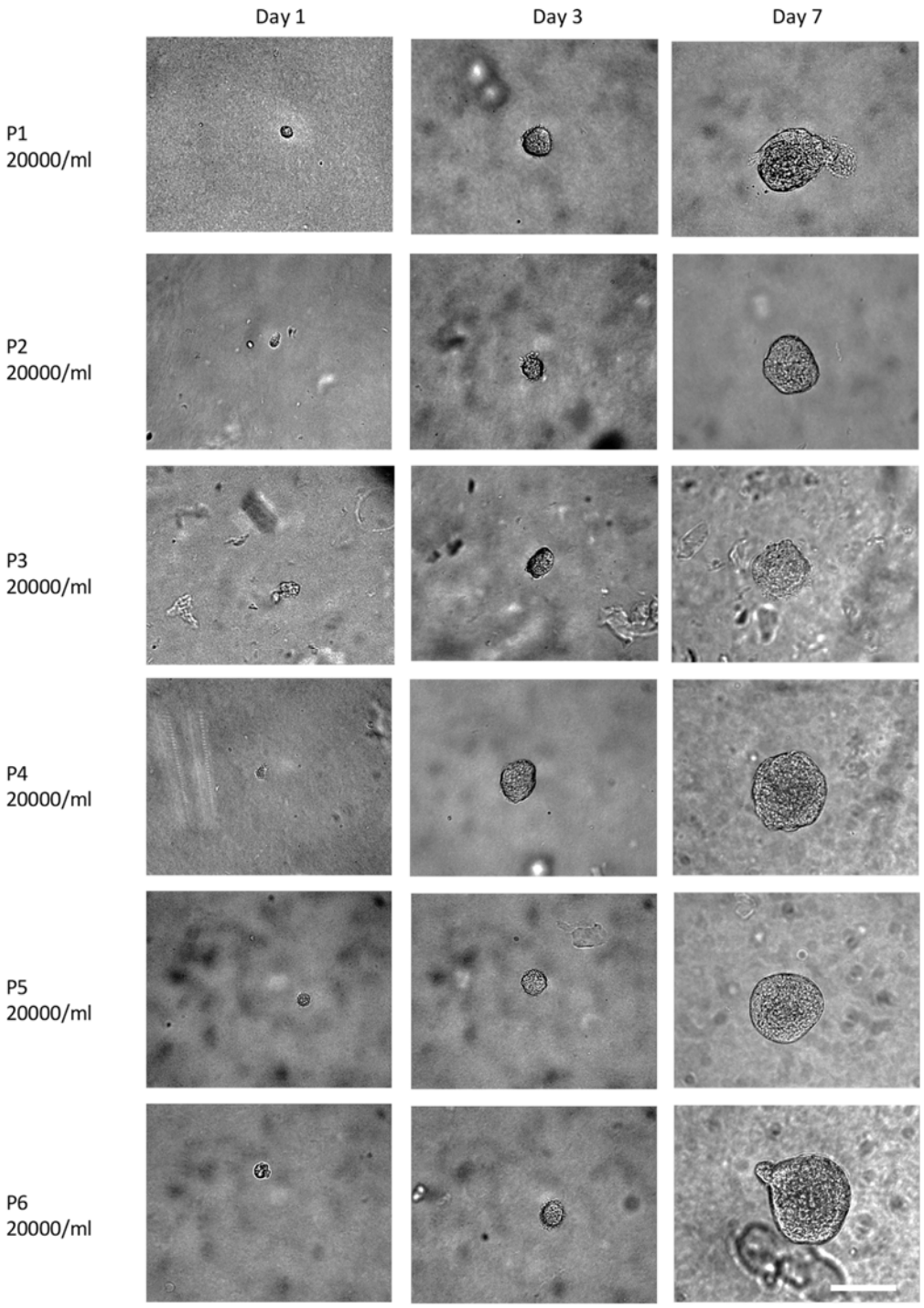
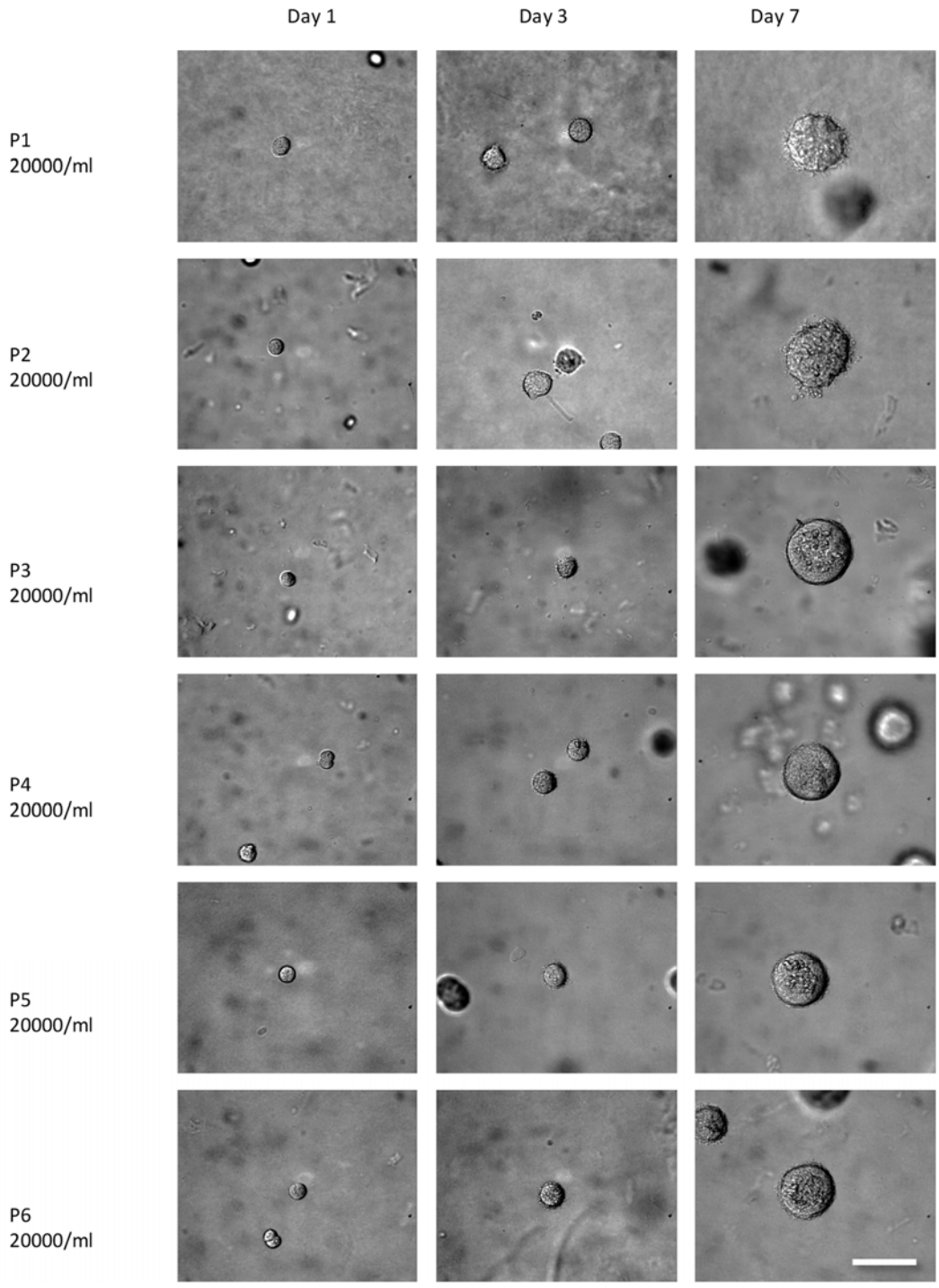
Supplementary Figures



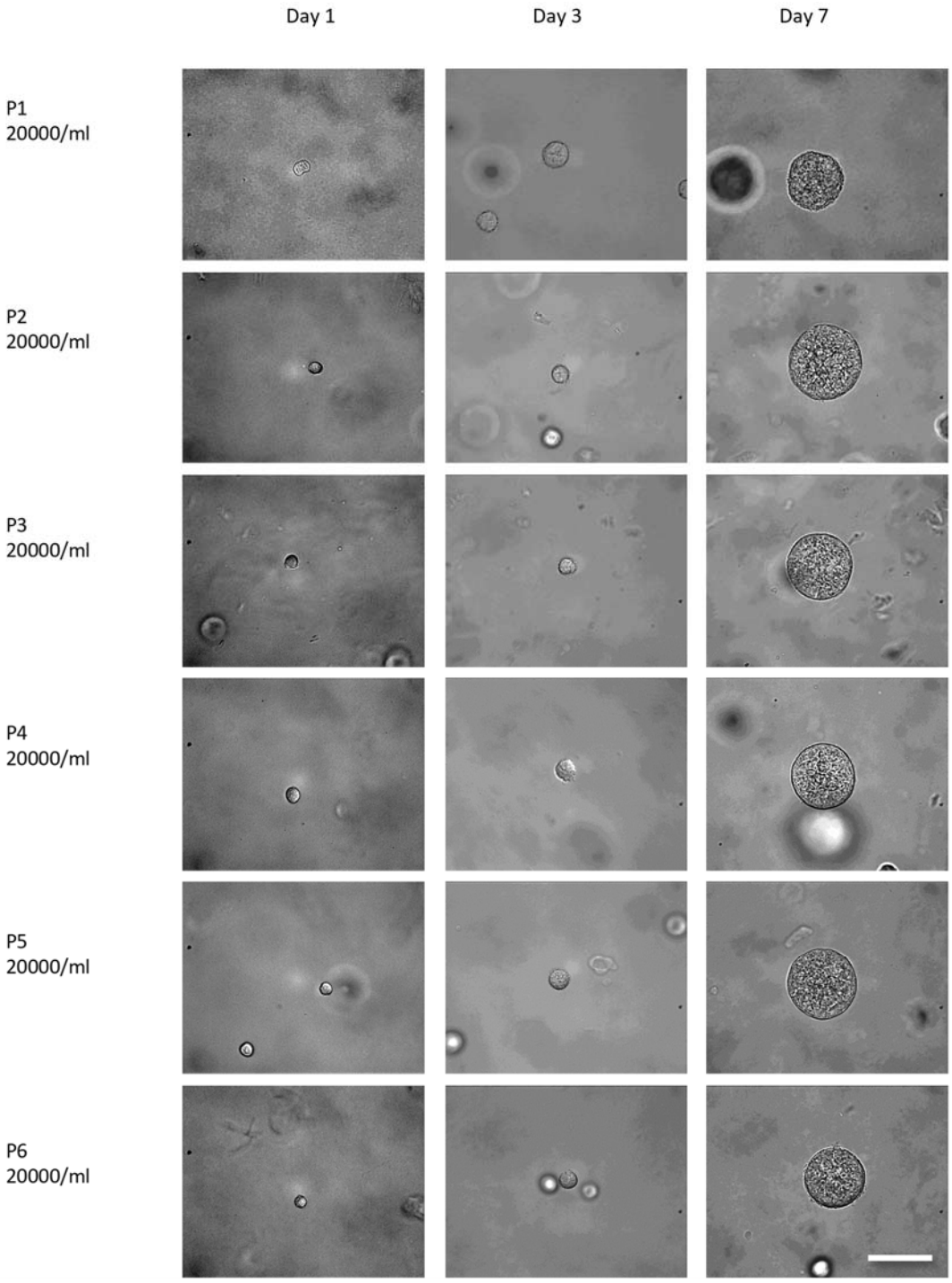
Supplementary Figure 1. Mechanical properties of the hydrogels. (a, c) Temperature ramps of **P1**-**P6** and **S1**-**S6**. All gels have a LCST at approximately 15°C. (b) Storage modulus of **P1-P6** gels before and after GRGDS conjugation. Before being functionalised with adhesive peptides, polymers with denser azide moieties (**P1, P2, P3**) formed relatively stiffer gels (500-700 Pa), compared with polymers with sparse azides (300-500 Pa). As a consequence of peptide addition by click reaction, stiffness of polymer gels with denser azide moieties decreased (**P1, P2, P3**). In contrast, **P4** showed an increase instead and **P5** remained the similar value in stiffness. It is likely that high density of additional peptides disrupts the integrity of polymer network and decrease the gel stiffness. (d) The K'- σ_c curves of **P1-P6** collapse to a single master curve when scaled to G' and σ_c , with a stiffening index m=1. (e) Correlation of storage moduli, G' and critical stress and σ_c of gels with the spacing of the peptide on the single polymer chain.



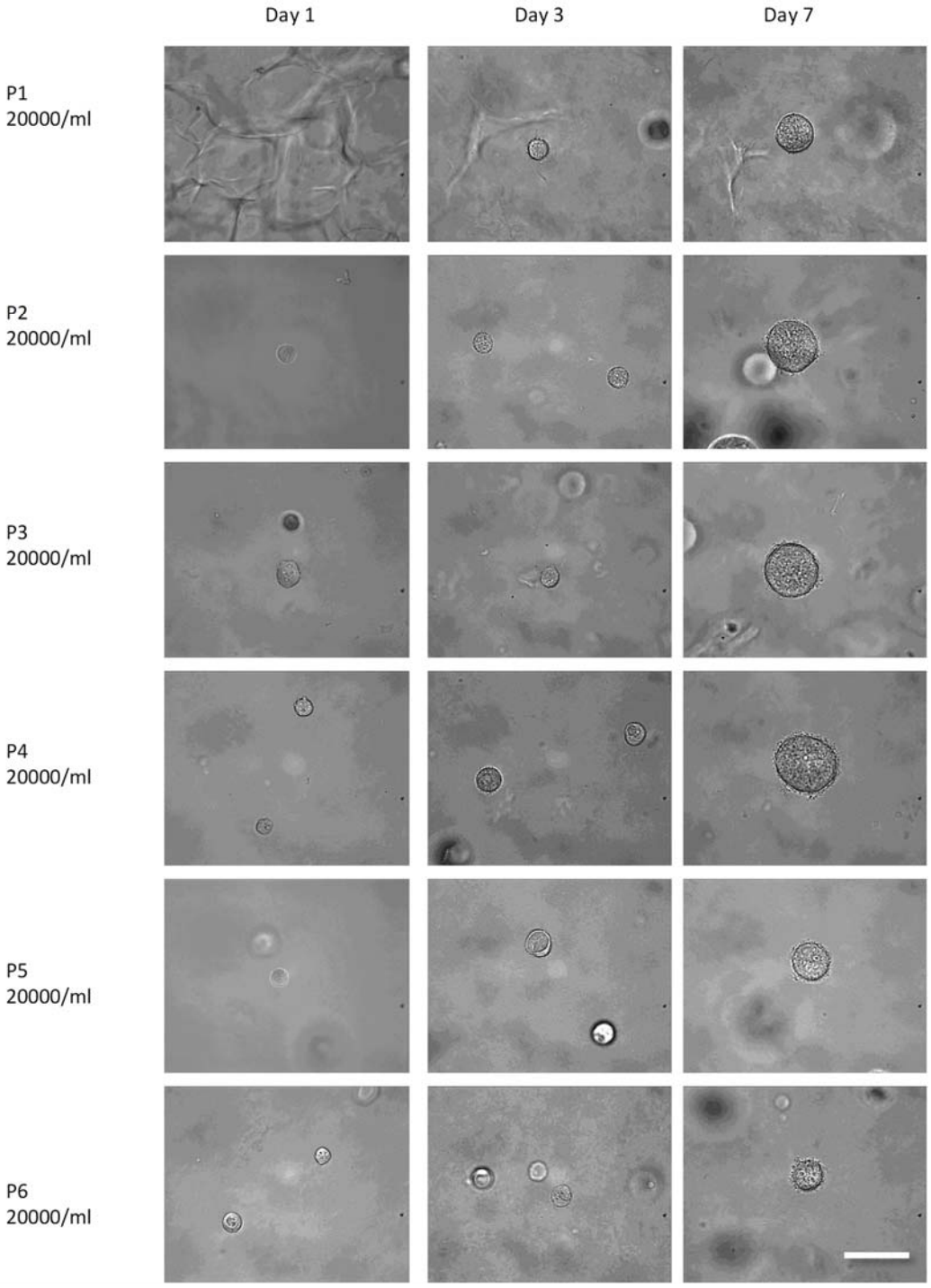
Supplementary Figure 2. Morphological change of human bladder smooth muscle cells (hbSMCs) with time in **P1**-**P6**. Scale bar: 70 μm.



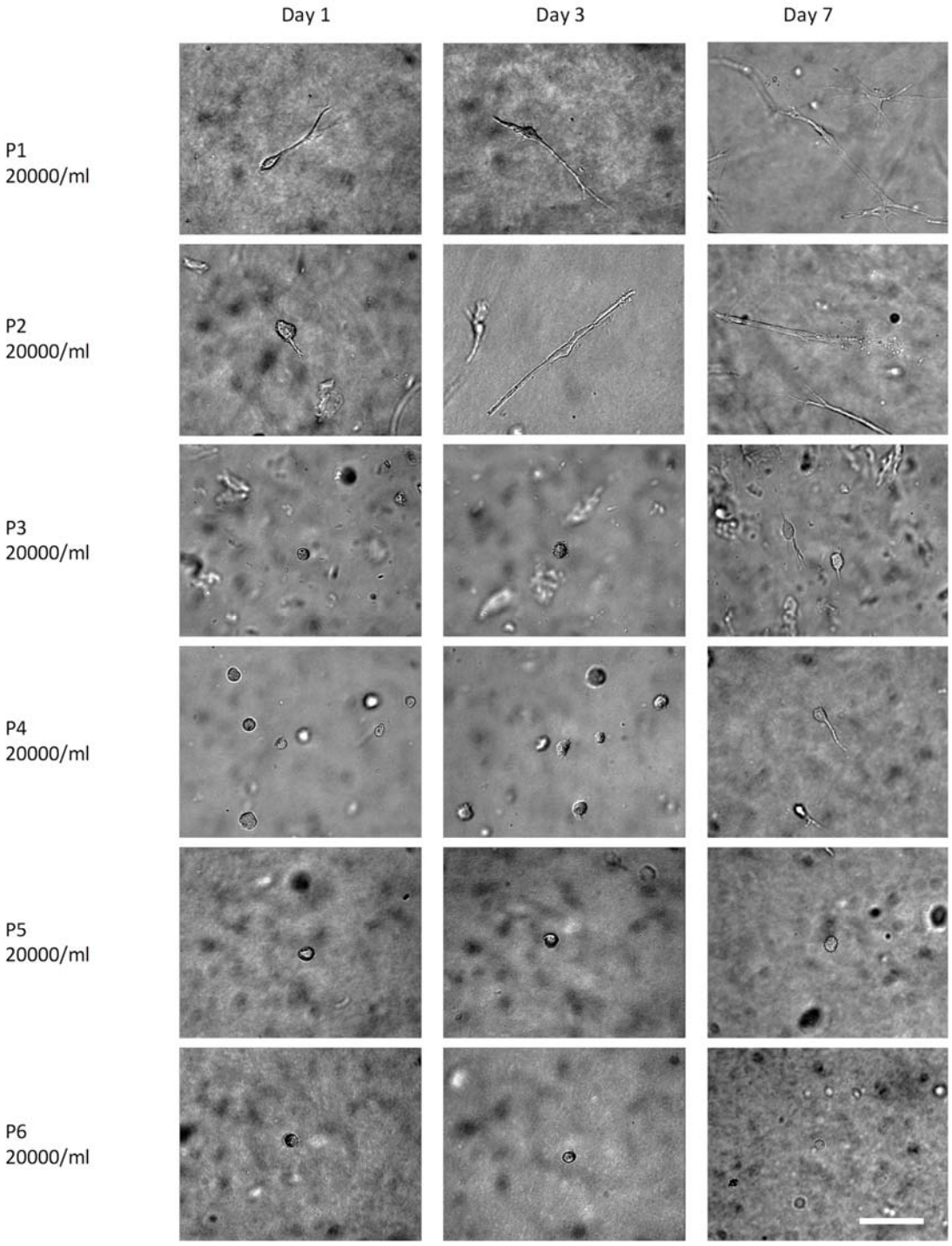
Supplementary Figure 3. Morphological change of HeLas with time in **P1**-**P6**. Scale bar: 70 μm.



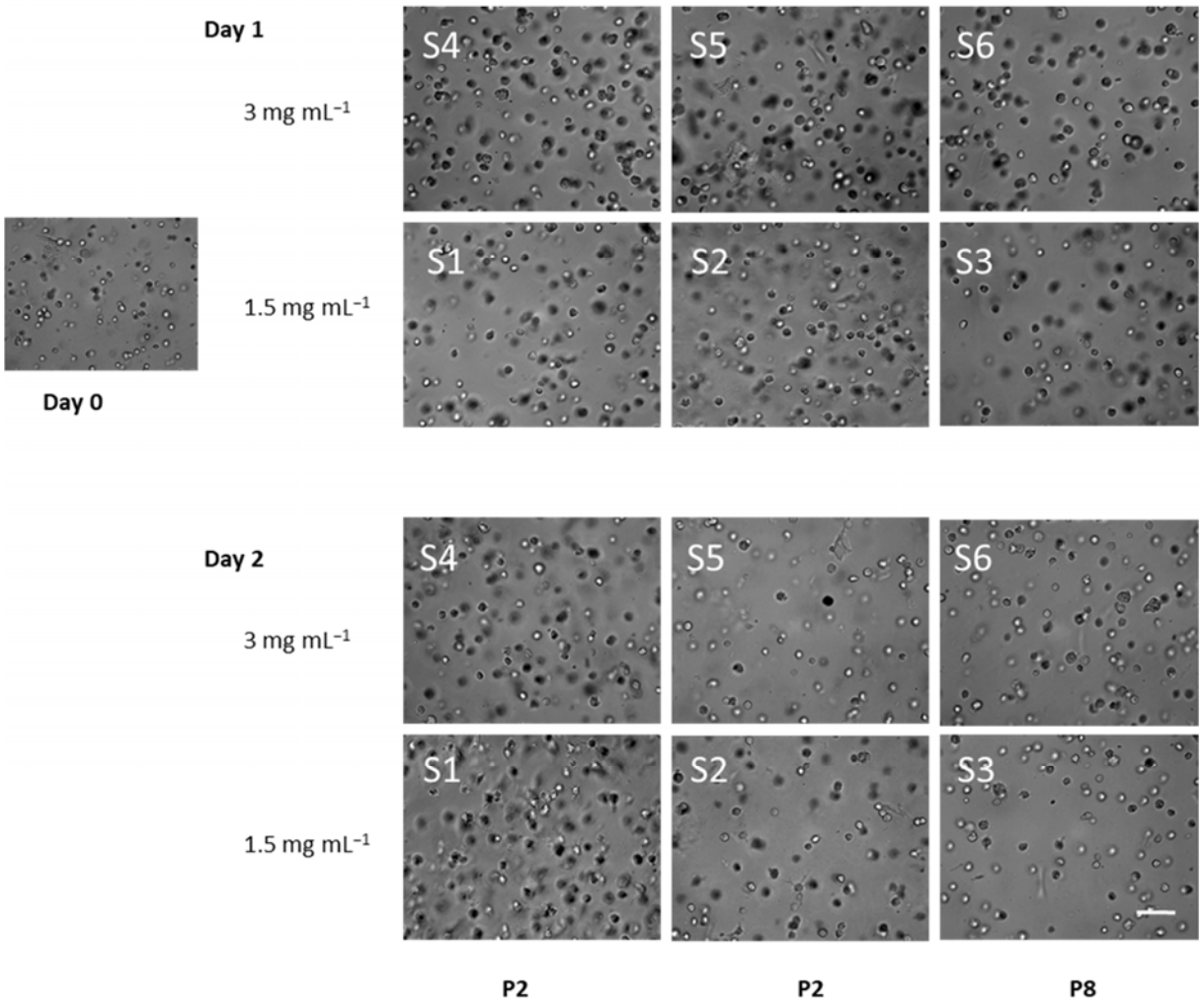
Supplementary Figure 4. Morphological change of T24 with time in **P1**-**P6**. Scale bar: 70 μm.



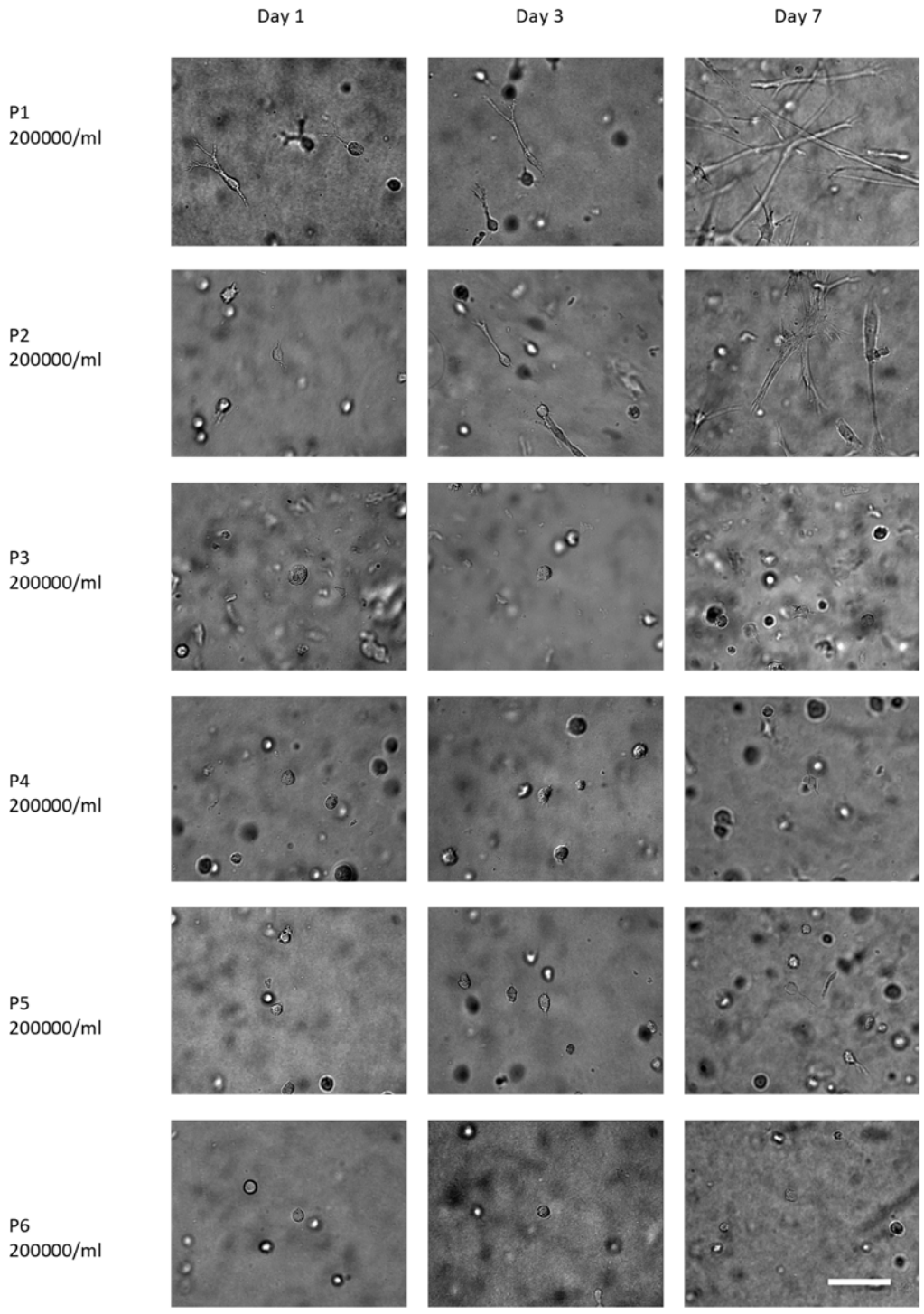
Supplementary Figure 5. Morphological change of SKRC52s with time in **P1**-**P6**. Note that fluorescence staining of the vessel-like structure proved that these patterns were not from cellular structures. We suppose that the phenomenon is due to optical reflection of cell-gel constructs. Scale bar: 70 μm.



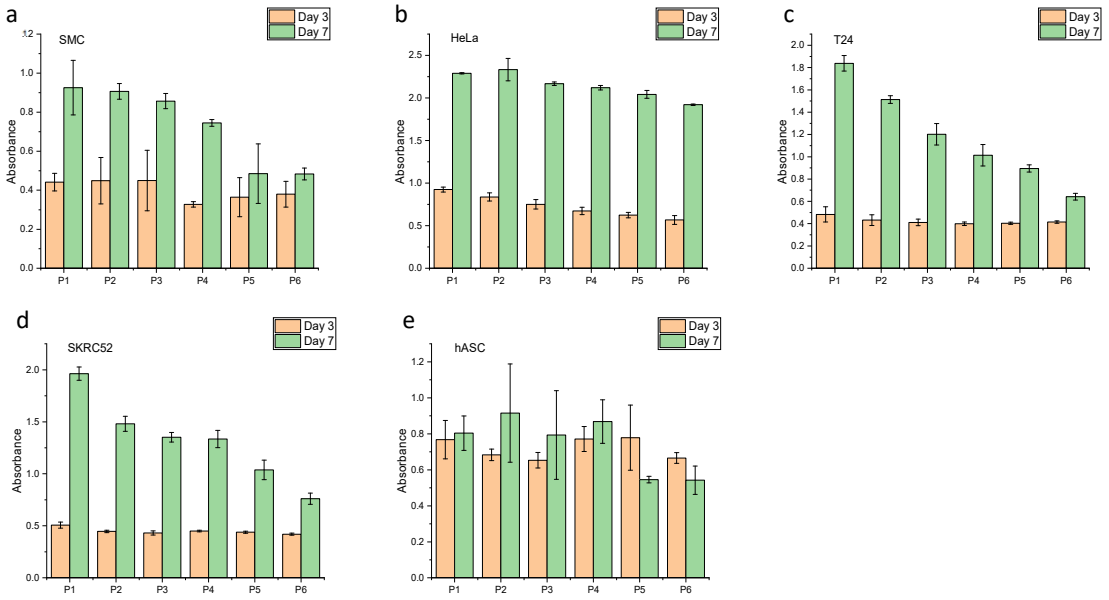
Supplementary Figure 6. Morphological change of hASCs with time in **P1**-**P6**. Scale bar: 70 µm.



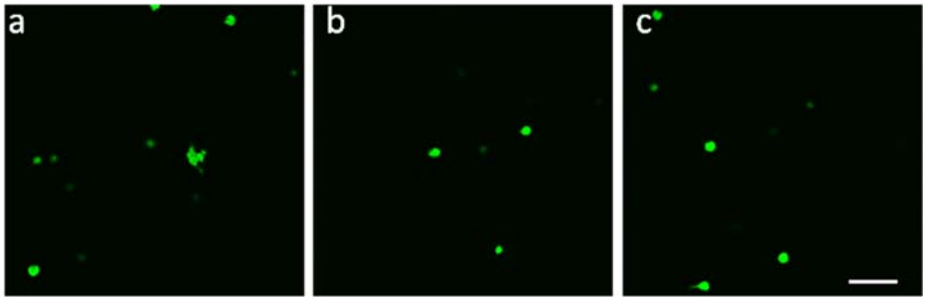
Supplementary Figure 7. Morphologies of hASCs at day 0, 1 and 2 in **S1**-**S6**. Scale bar: 70 µm.



Supplementary Figure 8. Morphological change of hASCs in **P1**-**P6** with a higher cell density (200,000 cells mL^{-1}). The cell response is similar with the lower density shown in the main text. Scale bar: 70 µm.



Supplementary Figure 9. Crude data of the WST-1 proliferation assay from Figure 4, showing the measured absorbance at λ = 450 nm without normalization is shown. (a, b, c, d, e) represent the proliferation of SMCs, HeLas, T24s, SKRC52s and hASCs respectively. Cell density: 20,000 cells mL^{-1}.



Supplementary Figure 10. Live/Dead staining of hASCs in **P2**, 48h after encapsulation. Three representative images at different gel areas prove that stem cells have high viability in PIC-RGD gels. Green: live cells. Red: dead cells. NB. no dead cell is observed in these images. Scale bar: 100 µm.

The staining protocol is adapted from the product manual (Invitrogen, Thermo Fisher, USA). In brief, cell culture medium on top of gels was gently removed. A 2 µM calcein AM and 4 µM EthD-1 working solution was prepared in α-MEM medium and added onto gel samples. After a 30-minute incubation in the cell incubator, all samples were washed by pre-warmed 0.9% NaCl and imaged by confocal fluorescence microscope.