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Supplementary Information

Enhanced Multi-Lineage Differentiation of Human Mesenchymal Stem/Stromal Cells within Poly (N-isopropylacrylamide-Acrylic Acid) Microgel-Formed Three-Dimensional Constructs

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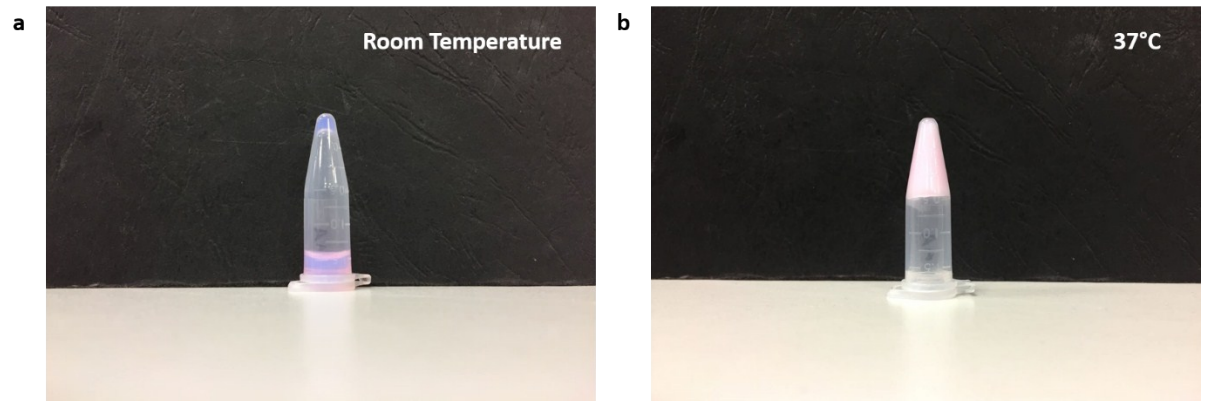

Figure S1 Thermosensitive behaviour of p(NIPAAm-AA) microgel-formed 3D constructs. (a) sol status at room temperature and (b) gel status at 37°C.

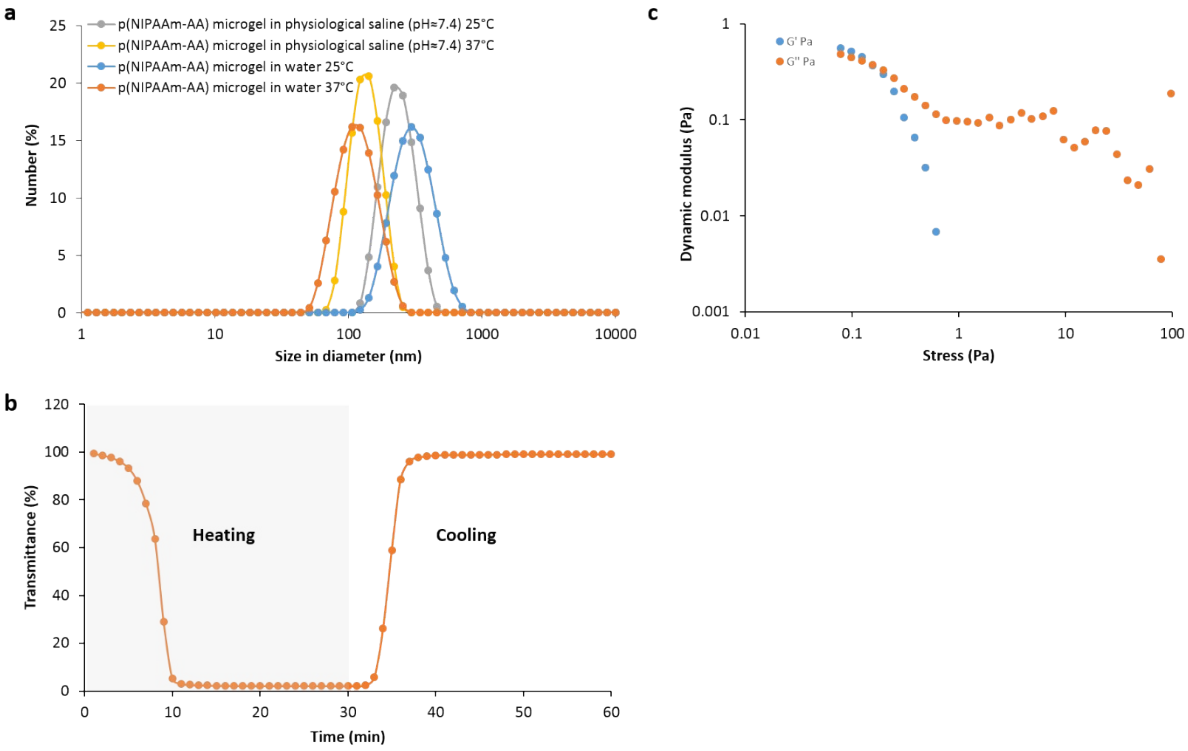

Figure S2 (a) Size distribution of 0.5 mg/mL p(NIPAAm-AA) microgels in Milli-Q® water or 1×DPBS (pH ≈ 7.4) at 25°C and 37 °C. (b) Time-dependent gelation process was investigated by measuring the transmittance (%) of the p(NIPAAm-AA) microgel-formed three-dimensional (3D) constructs heating from 0 to 30 min and cooling from 31 to 60 min with a wavelength of 720 nm light. (c) The stress dependence of the dynamic moduli (elastic modulus G' and viscous modulus G'') of 30 mg/mL p(NIPAAm-AA) microgel-formed constructs at 37 °C.

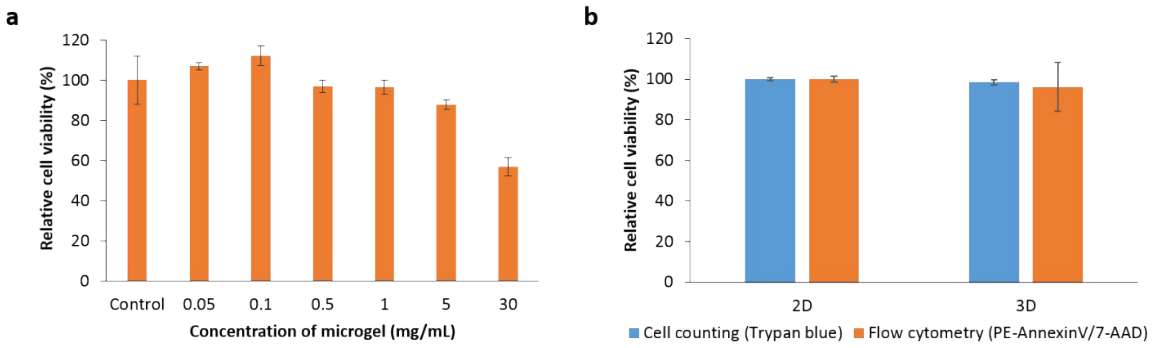

Figure S3 UE7T-13 cell viability assay after 24 hours. (a) Surface toxicity assay of various concentration of p(NIPAAm-AA) microgel by MTT. Control: without microgel. The MTT absorbance was normalized to the control and presented as relative cell viability. (b) 3D toxicity assay of microgel at the concentration of 30mg/mL by cell counting with trypan blue staining or flow cytometry with PE-AnnexinV and 7-AAD double staining. 2D: cells cultured on the multi-well plate; 3D: cells cultured within the thermosensitive p(NIPAAm-AA) microgel-formed constructs. The cell viability was normalized to 2D and presented as relative cell viability. Results were mean ± standard error (n=3). No significant difference was found between 2D and 3D.

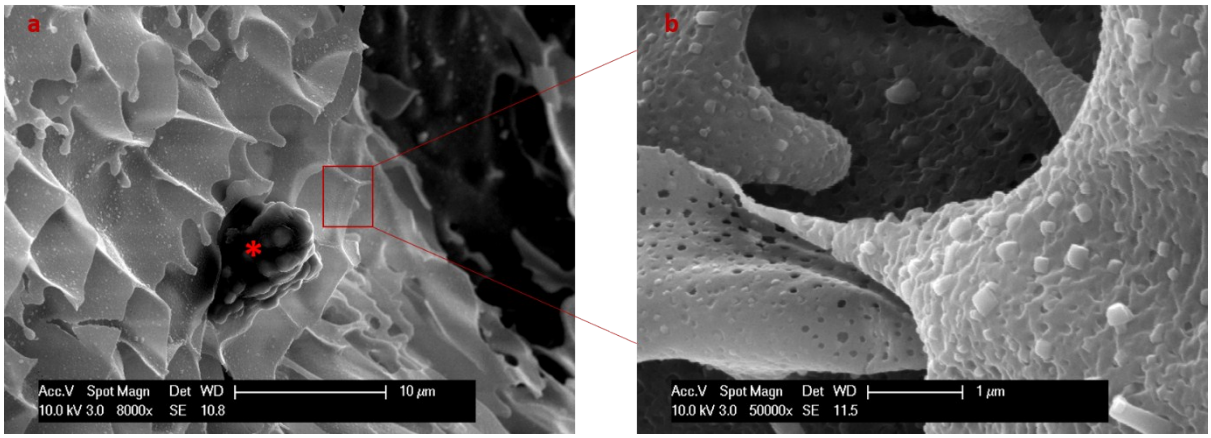

Figure S4 SEM images of the cross-sectional p(NIPAAm-AA) microgel-formed 3D construct. The microgel-formed constructs were quickly freezed in liquid nitrogen when at gel status and dried under vacuum. (a) UE7T-13 cells were cultured in the 3D construct on day 1. The asterisk showed an incorporated cell within p(NIPAAm-AA) microgel-formed 3D constructs. (b) Morphology of the 3D construct at higher magnification.

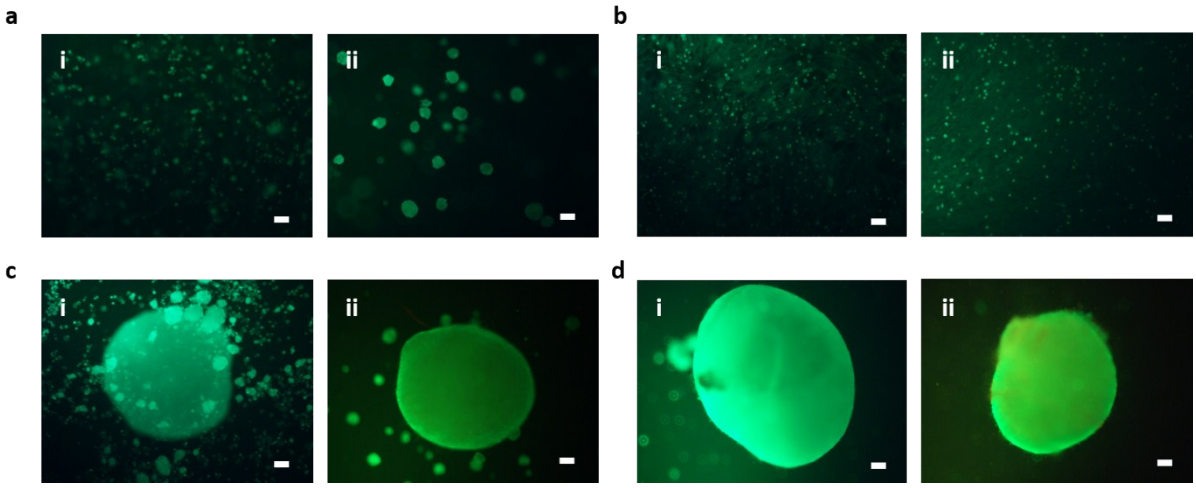

Figure S5 Fluorescent images of UE7T-13 cells cultured (a) in the thermosensitive p(NIPAAm-AA) microgel-formed 3D constructs with an additional layer of p(NIPAAm-AA) microgel coating on the bottom; (b) in the thermosensitive p(NIPAAm-AA) microgel-formed 3D constructs without p(NIPAAm-AA) coating; (c) on the low attachment surface; (d) in the polypropylene tube for (i) 1 day and (ii) 9 days by calcein AM and ethidium homodimer-1 double staining. Green: live cells; Red: dead cells. Scale bar: 100 μm.

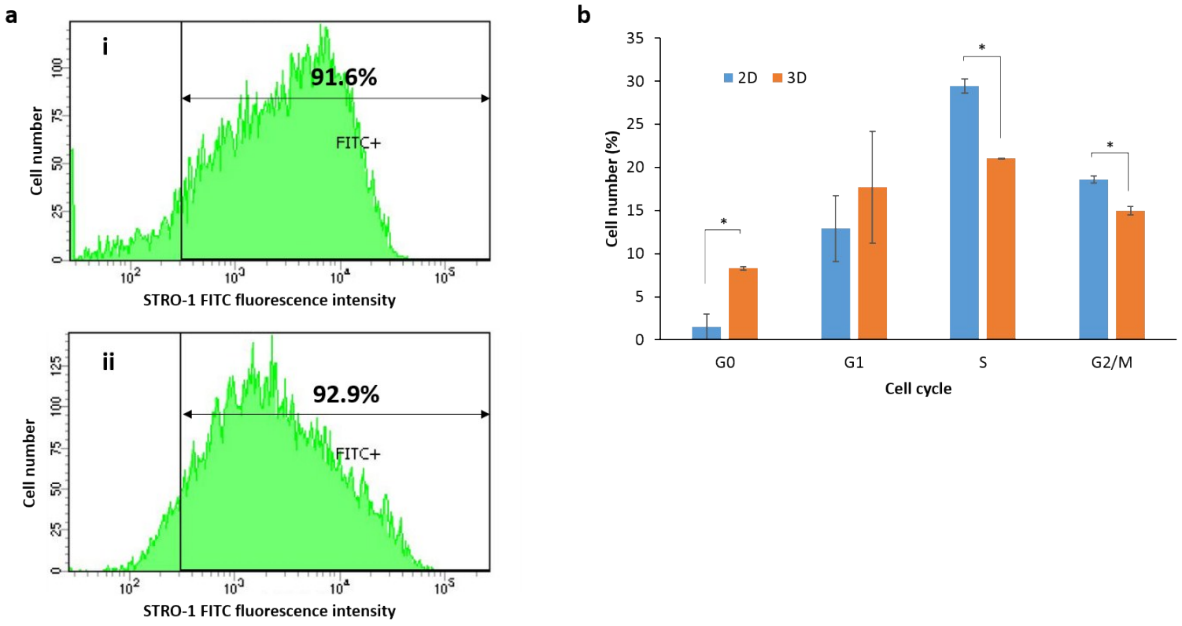

Figure S6 (a) Representative STRO-1 flow cytometry of UE7T-13 in (i) 2D and (ii) 3D. The percentage of fluorescein isothiocyanate (FITC)-labelled STRO-1 positive cells for 2D and 3D was 91.6% and 92.9%, respectively. (b) Cell cycle assay of UE7T-13 in 2D and 3D by FITC-labelled KI-67 antibody and propidium iodide (PI) double staining flow cytometry. Data was presented as the mean ± standard error (n=3). *$p < 0.05$. 2D: cells were cultured on the two-dimensional plates; 3D: Cells were cultured in the p(NIPAAm-AA) microgel-formed three-dimensional constructs.