Electronic Supplementary Material (ESI) for Biomaterials Science.

Supplementary Information for: *Dual-stage growth factor release within 3D protein-engineered hydrogel niches promotes adipogenesis*

Target	Forward Sequence (5' – 3')	Reverse Sequence (5' – 3')
GAPDH[1]	CATCAAGAAGGTGGTGAAGC	GTTGTCATACCAGGAAATGAGC
CEBPβ[1]	ACTTCAGCCCGTACCTGGAG	GAGAAGAGGTCGGAGAGGCAAGT
C/EBPα[1]	ACGATCAGTCCATCCCAGAGTA	GGCAAGTATCCGAGCAAAAC
PPARγ[1]	CCGTGGATCTCTCCGTAATG	ACTCTGGATTGAGCTGGTCG
FABP4[2]	GCTTTGCCACCAGGAAAGTG	ATGGACGCATTCCACCACCA
GLUT4[2]	GCCGGACGTTTGACCAGAT	TGGGTTTCACCTCCTGCTCTA
Leptin	ACGATCAGTCCATCCCAGAGTA	GGCAAGTATCCGAGCAAAAC
UCPI	ACTTCAGCCCGTACCTGGAG	GAGAAGAGGTCGGAGAGGCAAGT

Table S1: qPCR Primer Sequences. Forward and reverse primers selected for analysis of the following human mRNA genes: CCAAT/enhancer binding protein subunits beta and alpha (C/EBPβ, C/EBPα), Peroxisome proliferator-activated receptor gamma (PPARγ), fatty acid binding protein (FABP4), glucose transporter 4 (GLUT4), Leptin, and Uncoupling protein 1 (UCP1), relative to the housekeeping gene, Glyceraldehyde 3-phosphate dehydrogenase (GAPDH). Sequences were designed using PrimerQuest Tool (IDT) with previously reported cDNA clones for each target.[1-3]

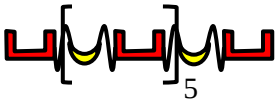
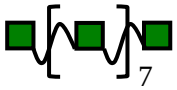
MITCH Components	Illustration	
C7		MGSSHHHHHHSSGLVPRGSSSGHIDDDDKVDGT [RLPAGWEQRMDVKGRPYFVDHVTKSTTWEDPRPE]GTLDEL [AGAGAGPEG]$_2$RGDSAGPEG[AGAGAGPEG]$_2$ELLDGT ([RLPAGWEQRMDVKGRPYFVDHVTKSTTWEDPRPE]GTLDEL [AGAGAGPEG]$_2$[RGDSAGPEG][AGAGAGPEG]$_2$ ELLDGT)$_5$ [RLPAGWEQRMDVKGRPYFVDHVTKSTTWEDPRPE]GTLE
P9		MGSSHHHHHHSSGLVPRGSSSGHIDDDDKVDGT [EYPPYPPPPYPSG]GTLDEL[AGAGAGPEG]$_2$ELLDGT ([EYPPYPPPPYPSG]GTLDEL[AGAGAGPEG]$_2$ELLDGT)$_7$ [EYPPYPPPPYPSG]GTLE
Peptide Domains	**Illustration**	
CC43 WW		RLPAGWEQRMDVKGRPYFVDHVTKSTTWEDPRPE
Proline-rich Peptide		EYPPYPPPPYPSG
Hydrophilic Spacer		[AGAGAGPEG]$_2$
Cell-attachment Ligand		RGDSAGPEG

Table S2: Molecular design of MITCH. Illustrations and amino acid sequences of MITCH components C7 and P9, and constituent peptide domains contained with the full-length recombinant protein polymers.[4]

Supplementary Figures

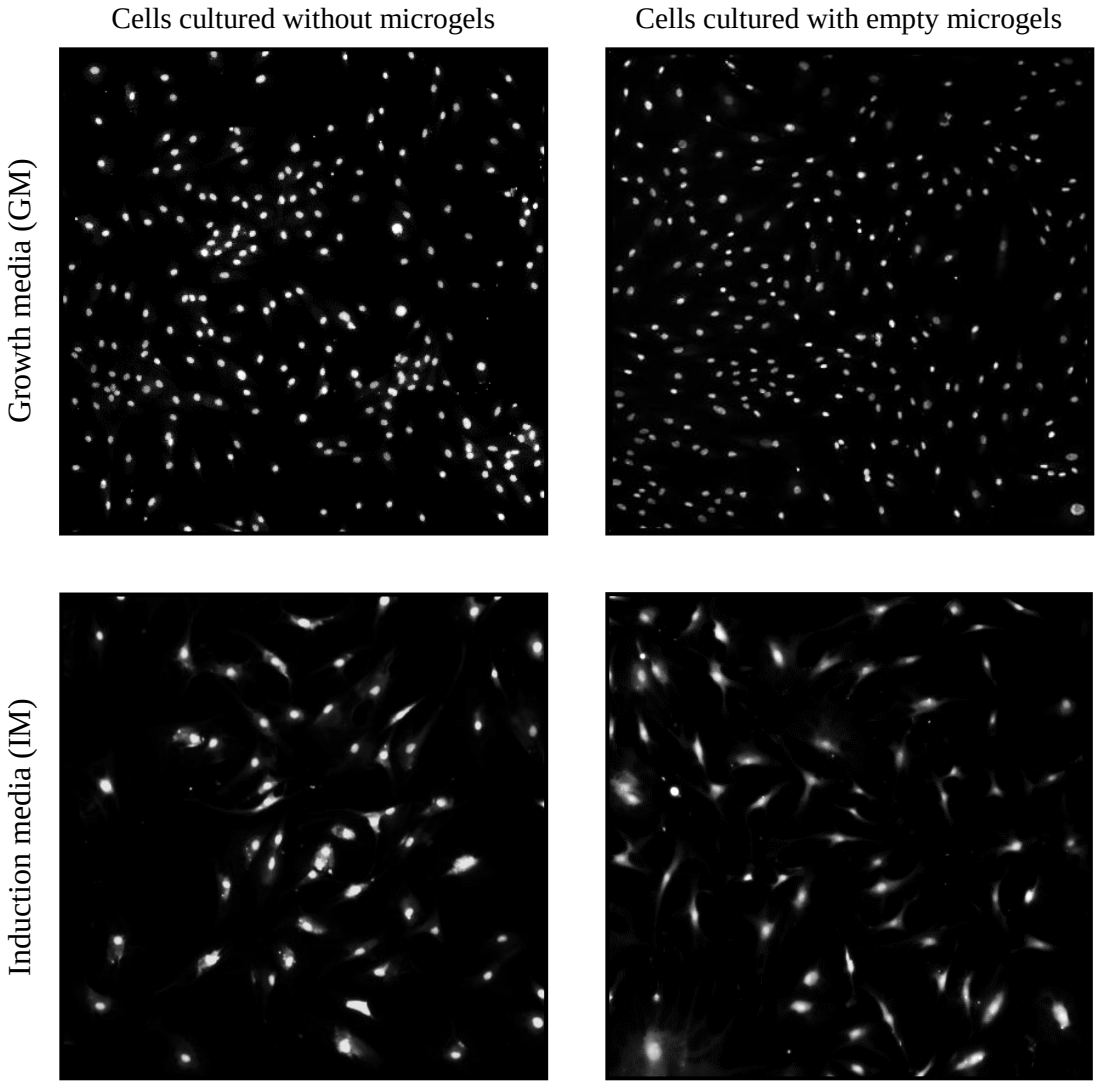


Figure S1. Representative images of hADSCs cultured on TCPS. Cell nuclei shown by DAPI staining and fluorescence microscopy show no differences between cells cultured with and without microgels. Cell counts from DAPI staining are as follows: GM without microgels (5.87×10^7 cells/well) and with empty microgels (6.11×10^7 cells/well), IM without microgels (3.65×10^7 cells/well) and with empty microgels (4.02×10^7 cells/well).

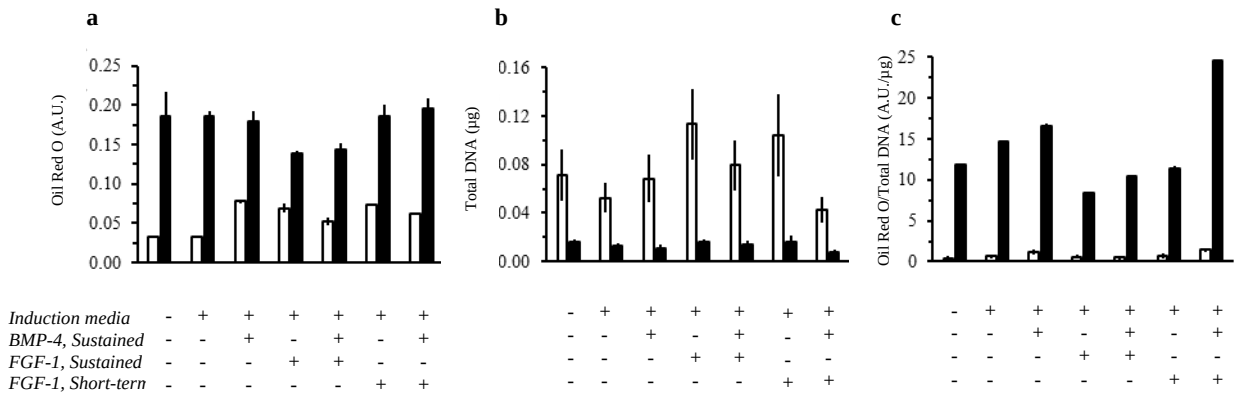


Figure S2. Comparison of *in vitro* adipogenesis of hADSCs cultured on 2D TCPS and in 3D MITCH at day 7. (a) Total lipid accumulation was estimated by Oil Red O quantification. (b) The total number of cells was estimated by quantifying total dsDNA. (c) The estimated lipid accumulation per cell was determined by normalizing Oil Red O absorbance to total dsDNA. All differences between 2D TCPS and 3D MITCH cultures are statistically significant, $p < 0.05$.

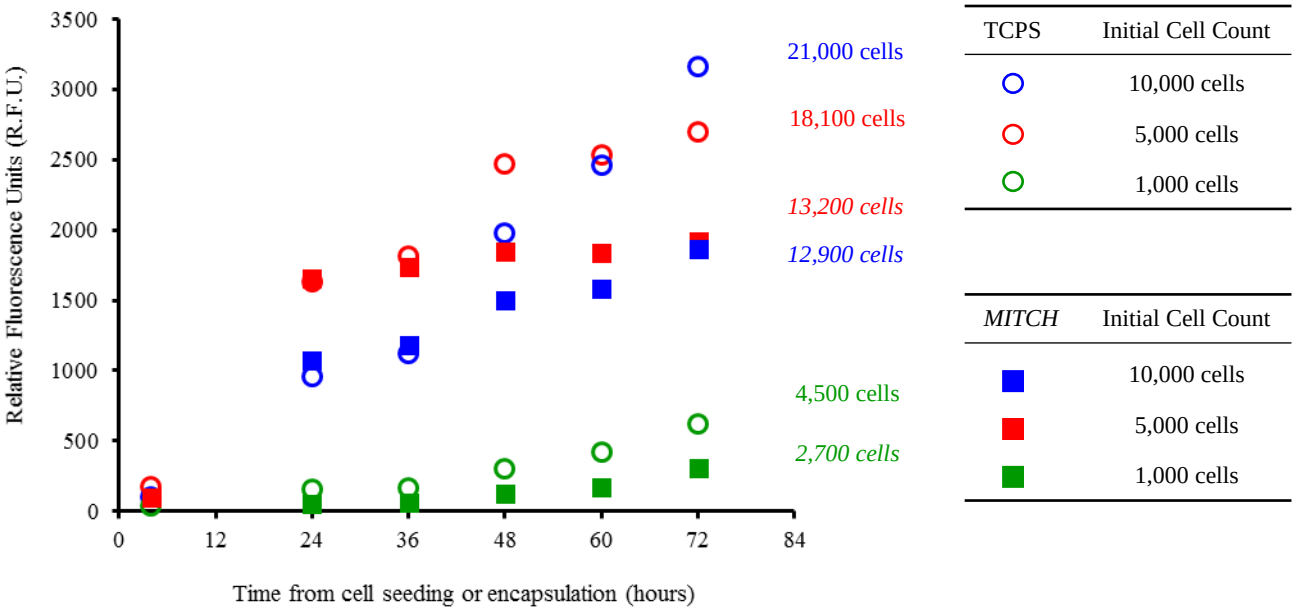


Figure S3. Comparison of hADSC proliferation between 2D and 3D cell cultures. Viable cell activity and proliferation was estimated using PrestoBlue® reagent per the manufacturers' instructions (Life Technologies). Using a standard curve across the range of 500 to 50000 cells, R.F.U. was converted to the number of cells: *Number of cells = (R.F.U. + 97.55)/0.1546*, $R^2 = 0.9933$. Cell counts following 3 days of proliferation are reported above. Cell proliferation is reduced when cells are cultured within 3D MITCH.

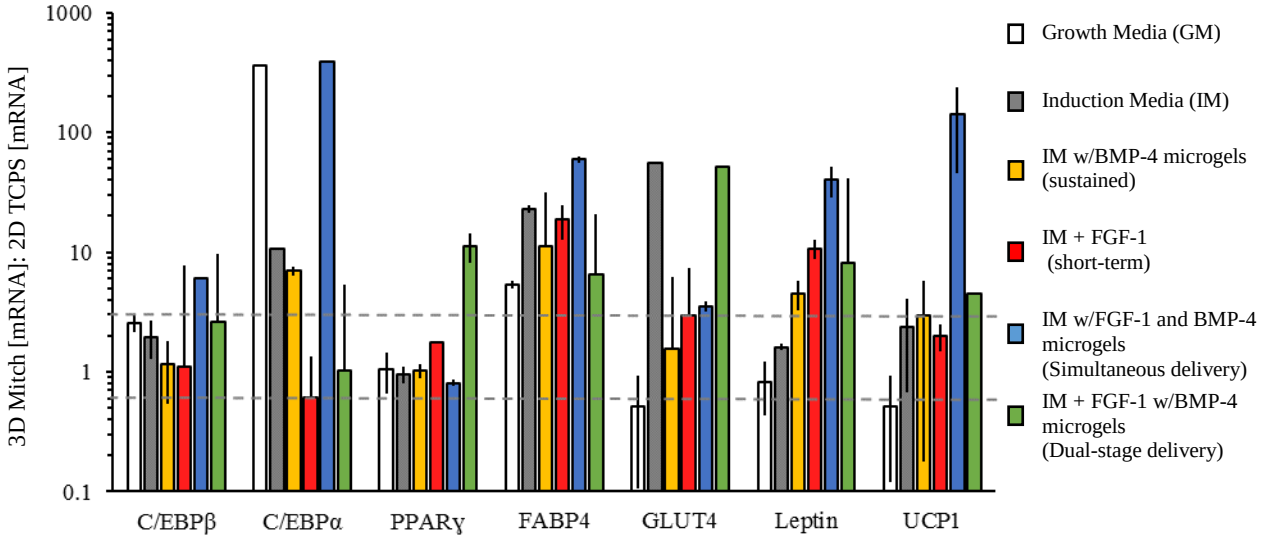


Figure S4. Fold-change in mRNA expression for cells cultured in 3D MITCH normalized to 2D TCPS. Dashed lines indicate a 2-fold increase or decrease in mRNA concentration, demonstrating that mRNA concentrations is either comparable or significantly increased when cells are cultured within MITCH.

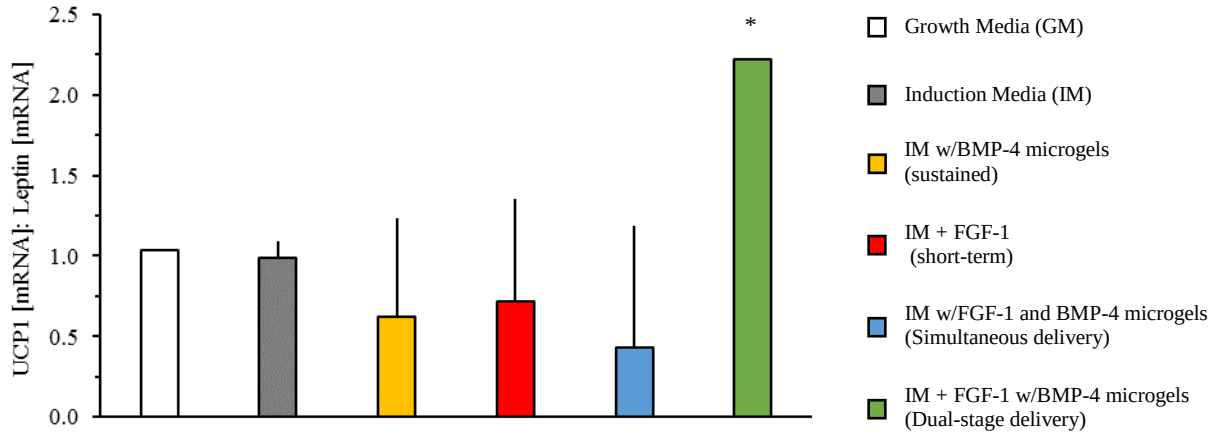


Figure S5. Ratio of UPC1 mRNA concentration over Leptin mRNA concentration. The relative mRNA concentration reported in 3D MITCH cultures was used to calculate the ration of UCP1 to Leptin mRNA concentration, * $p < 0.05$. UCP1 and Leptin are expected to be upregulated in brown and white adipocytes, respectively.[5]

References

1 S. W. Qian, Y. Y. Zhang, H. Y. Huang, Y. Liu, X. Sun *et al.*, *BMC Dev Biol.*, 2010, **10**, 47.
2 C. D. Wrann, J. Eguchi, A. Bozec, Z. Xu, T. Mikkelsen *et al.*, *J Clin Invest.*, 2012, **122**, 1010.
3 H. S. Sacks, J. N. Fain, S. W. Bahouth, S. Ojha, A. Frontini *et al.*, *J Clin Endocrinol Metab.*, 2013, **98**, E1448.
4 C. T. Wong Po Foo, J. S. Lee, W. Mulyasasmita, A. Parisi-Amon, and S. C. Heilshorn, *Proc Natl Acad Sci U S A.*, 2009, **106**, 22067.
5 S. Cinti, *Ann Med.*, 2001, **43**, 104.