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Sequential multi-material 3D bioprinting of dual-stiffness peptide scaffolds for compartmentalized, multicellular bone marrow niche modeling

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Sequential multi-material 3D bioprinting of dual-stiffness peptide scaffolds for compartmentalized, multicellular bone marrow niche modeling

Running title: Bioprinted multi-component bone marrow model

Table S1. **TaqMan probes**

Gene	Catalog number of TaqMan probe	Catalog number
ALPL	Hs00758162_m1	4331182
Osteocalcin (BGLAP)	Hs01587814_g1	4331182
COL1A1	Hs00164004_m1	4331182
Runx2	Hs01047973_m1	4331182
SP7	Hs01866874_s1	4331182
GAPDH	Hs99999905_m1	4448490

Table S2. Tabulated measurements of G', G", and Tan δ at each concentration for both peptides

Peptide	Concentration (mg/mL)	Storage Modulus (G') (kPa)	Loss Modulus (G") (kPa)	Tan δ (G"/G')
IIFK	1	1.04 ± 0.166	0.204 ± 0.086	0.20
	2	5.19 ± 0.898	0.659 ± 0.159	0.13
	3	15 ± 2.88	1.49 ± 0.329	0.10
	6	43.6 ± 6.34	4.08 ± 0.497	0.09
IIZK	0.5	1.06 ± 0.129	0.315 ± 0.071	0.30
	1	3.52 ± 0.342	0.542 ± 0.084	0.15
	2	7.92 ± 0.559	1.03 ± 0.01	0.13
	3	11.4 ± 2.53	1.43 ± 0.35	0.13
	6	123 ± 11.1	14.1 ± 1.33	0.11

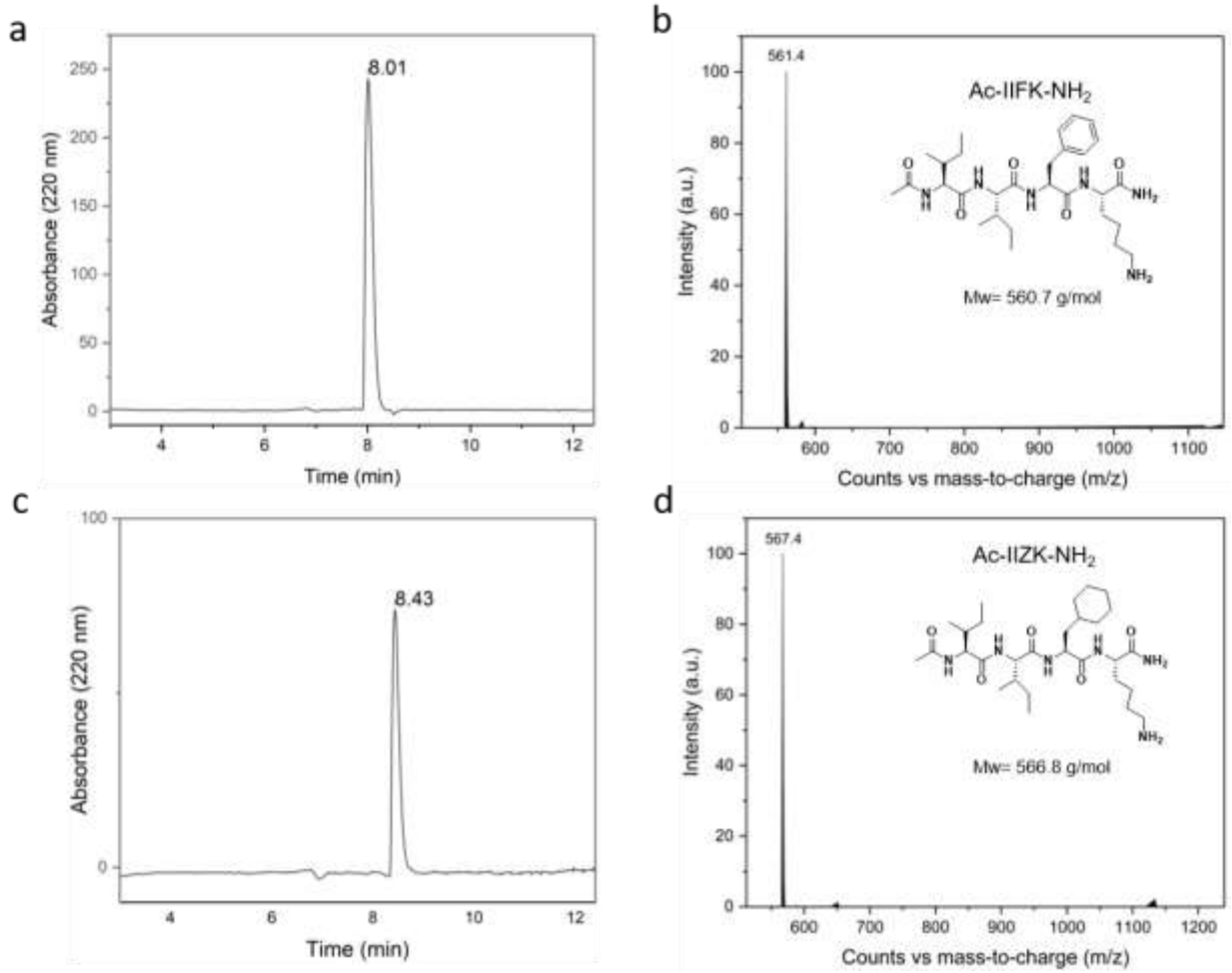

Figure S1. Analytical characterization of the peptides IIFK (Ac-IIFK-NH_2) and IIZK (Ac-IIZK-NH_2). (a) Chromatogram of IIFK at 220 nm, (b) mass spectrum of IIFK (calculated 560.7), $[M+H]^+$ observed 561.4, (c) chromatogram of IIZK at 220 nm, and d) mass spectrum of IIZK (calculated 566.8), $[M+H]^+$ observed 567.4.

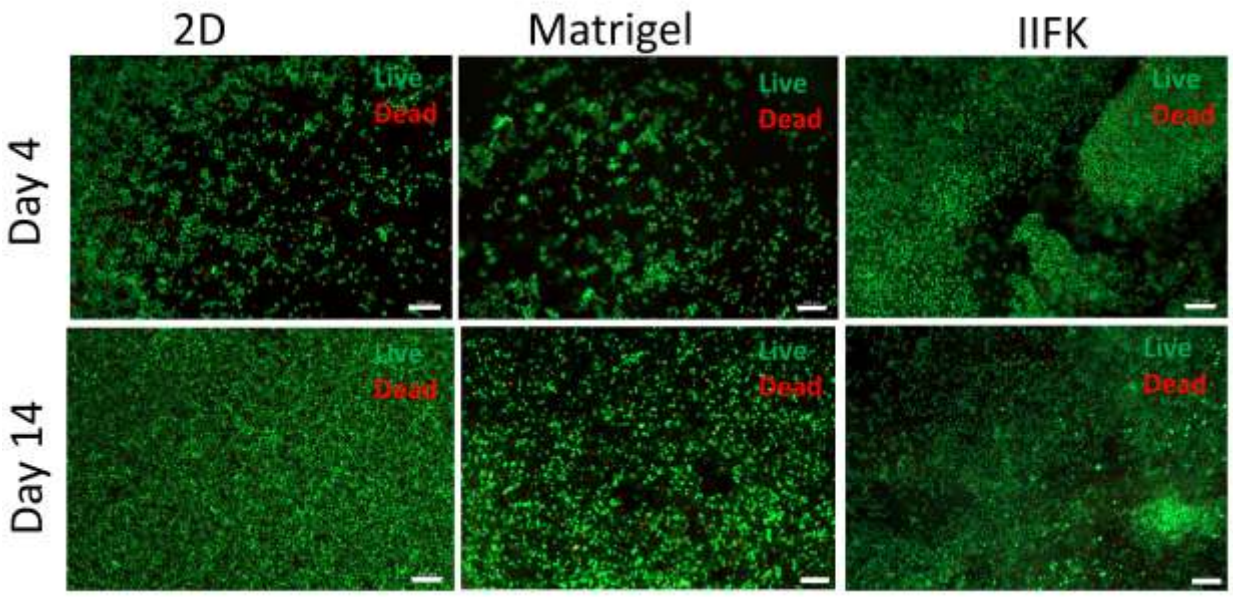

Figure S2. Cell viability assessment of HL-60 cells after 4 and 14 days of 3D culture within IIFK peptide hydrogel compared to 2D and Matrigel cultures. Cells were stained with calcein-AM (green, live cells) and ethidium homodimer-1 (red, dead cells). Scale bar: 100 µm.

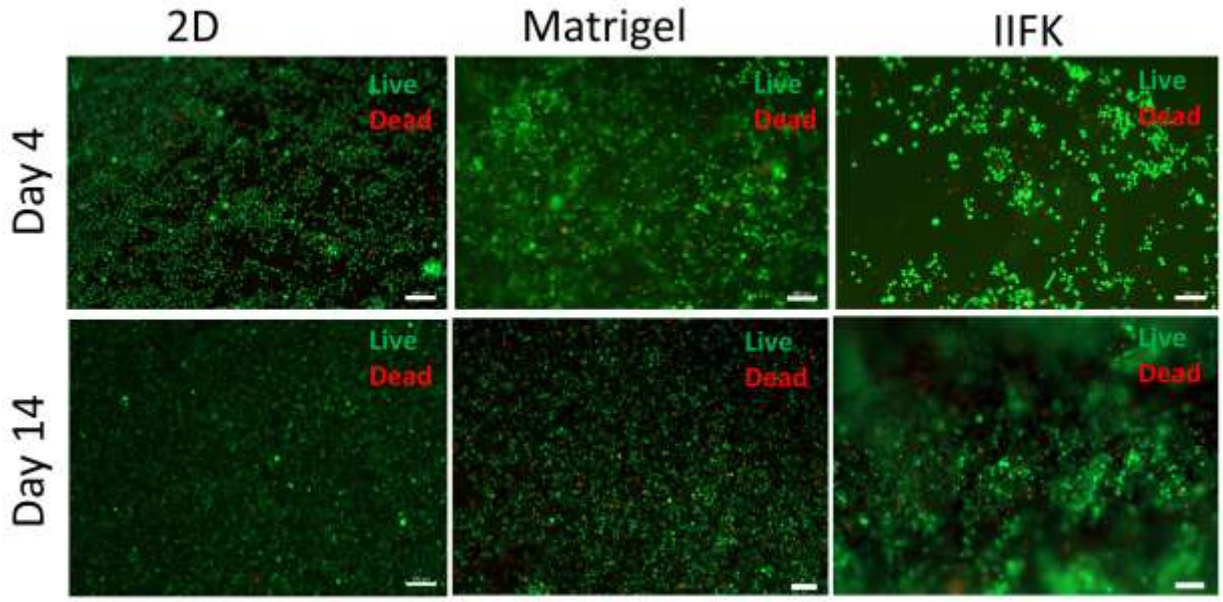

Figure S3. Cell viability assessment of KG-1a cells after 4 and 14 days of 3D culture within IIFK peptide hydrogel compared to 2D and Matrigel cultures. Cells were stained with calcein-AM (green, live cells) and ethidium homodimer-1 (red, dead cells). Scale bar: 100 µm.

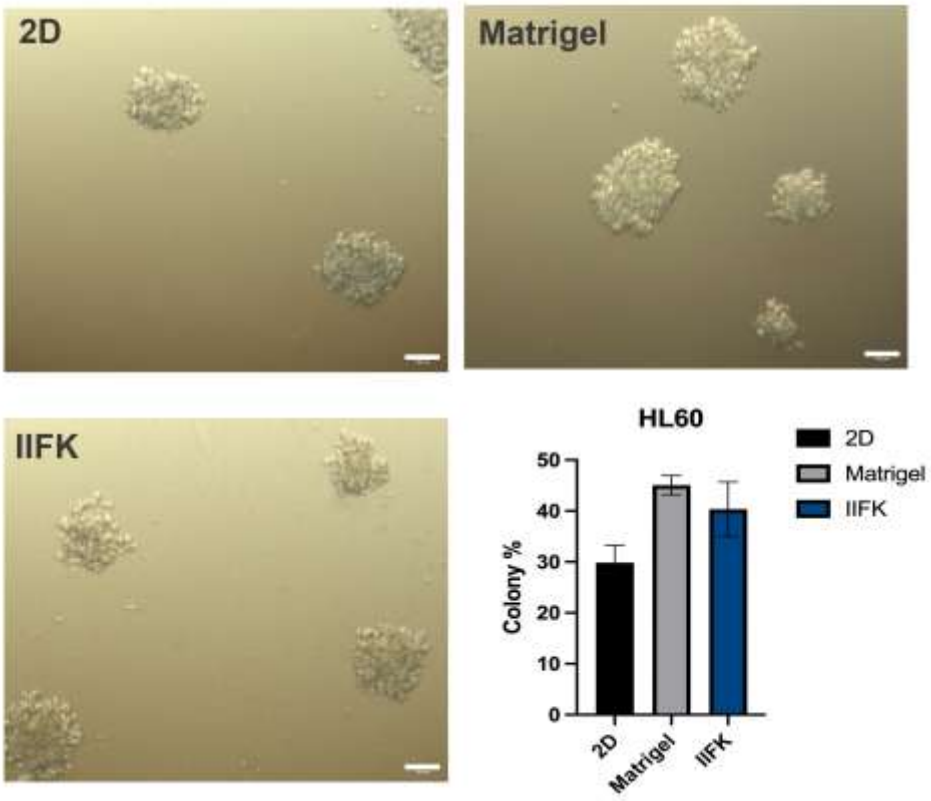

Figure S4. Assessment of clonogenicity potential of HL-60 cell line after 3D culture within IIFK peptide hydrogel compared to 2D and Matrigel culture. Light microscopy images of colony formation using methylcellulose media for 2D, Matrigel, and 3D cultured cells. Quantitative analysis shows colony counts for different culture conditions. Scale bar: 100 µm.

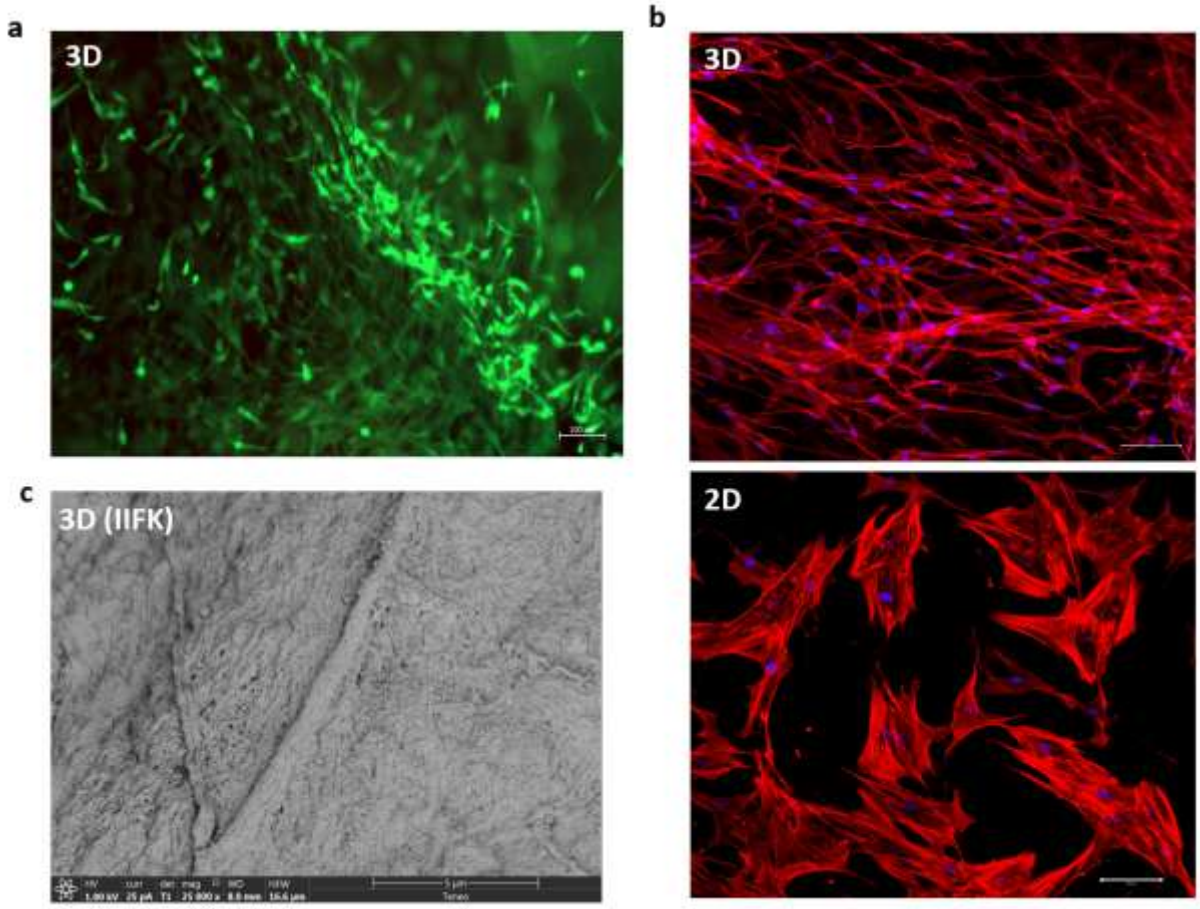

Figure S5. Assessment of BM-MSCs (Bone Marrow Mesenchymal Stem Cells. (a) Live/dead staining of IIFK-cultured cells. (Scale bar 100 μm). (b) Immunofluorescence cytoskeleton staining comparing IIFK 3D culture and 2D culture conditions, demonstrating cell elongation within the 3D culture. F-actin was stained with phalloidin (red) and the nucleus with DAPI (blue). (Scale bar 100 μm). (c) Scanning electron microscopy (SEM) images of cells in 3D culture within IIFK peptide hydrogel, demonstrating cell-matrix interactions.

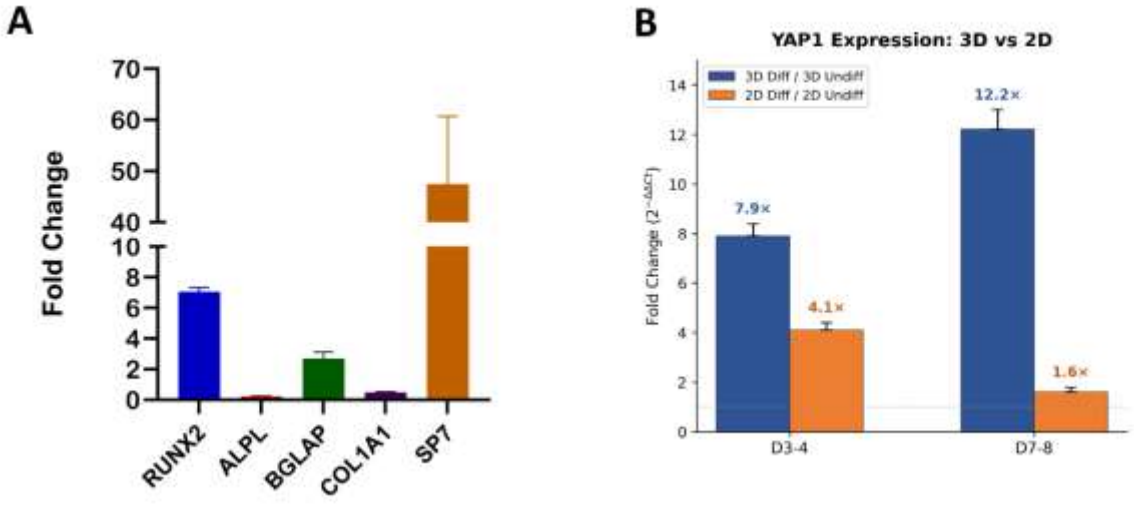

Figure S6. Gene expression analysis of mesenchymal stem cells (MSCs) across culture conditions. (A) Fold change of osteogenic-related genes in 3D cultured undifferentiated cells relative to 2D cultured undifferentiated cells at day 21. (B) Comparison of YAP1 expression between 3D and 2D culture systems during osteogenic differentiation. Fold change of YAP1 in osteogenic-differentiated cells relative to undifferentiated controls within each culture system at days 3–4 and 7–8, calculated using the 2^{-DDct} method with GAPDH as endogenous control. Error bars represent +SEM. YAP1 upregulation was substantially higher in 3D cultures (12.2-fold at days 7–8) compared to 2D cultures (1.6-fold), consistent with enhanced mechano-transduction signaling in the 3D peptide scaffold environment.

Supplementary videos

Videos S1. Printability of the IIZK peptide at 10 mg/mL peptide concentration

Videos S2. Printability of the IIFK peptide at 10 mg/mL peptide concentration