Supplementary Information

Supplementary Methods

Cell culture. Human bone marrow MSCs were obtained as a generous gift from Arnold Caplan Lab, Case Western Reserve University. Human adipose-derived stem cells (hADSCs) were isolated as previously described and received via a material transfer agreement[1S]. Bone marrow derived goat MSCs were harvested and expanded as previously described[2S]. Cells were cultured in expansion media on 2D surfaces while in cell- differentiation media in 3D gels. All constructs and substrates were cultured at 37 °C with 5% CO_2, and the media were changed every 2 to 3 days until harvesting. The formulations of various media are listed as follows:

Media	Ingredients
Growth media-hMSCs	DMEM (High glucose, 1 x), FBS 10%, Penicillin/Streptomycin (1% v/v), Glutamax (1% v/v), bFGF (8 ng/mL)
Adpogenic-hADSCs	DMEM (High glucose, 1 x), FBS 10%, Penicillin/Streptomycin (1% v/v), Dexamethasone (1 μM), Indomethacin/IBMX solution (100 μM/500 μM), Bovine insulin (10 μg/mL)
Osteogenic-hADSCs	DMEM (High glucose, 1x), FBS 10%, Penicillin/Streptomycin (1% v/v), Ascorbic Acid-2-phosphate (50 μM), Dexamethasone (100 nM), β-glycerophosphate (10 mM)
Chondrogenic-gMSCs	DMEM (High glucose, 1 x), FBS 10%, Dexamethasone (100 nM), Penicillin/Streptomycin (1% v/v), Sodium Pyruvate (100 μg/mL), L-Proline (40 μg/mL), Ascorbic Acid-2-phosphate (50 μg/ mL), Insulin, Transferrin, Selenous Acid (ITS) (1% v/v)

Gene-expression. (For all samples except for the study of chondrogenesis) The mRNAs of cells were extracted from either 3D hydrogels or 2D substrates with Trizol reagent (Invitrogen). Briefly, the mRNA was extracted with 1 mL Trizol per construct and then

precipitated, washed and resuspended in isopropanol, 75% ethanol, and DEPC H_2O, respectively. This suspension of mRNA in DEPC H_2O was incubated at 60 °C for 10 min and quickly put on ice. The concentration of mRNA was quantified using a DU500 UV-Vis Spectrophotometer (Beckman). The cDNA was synthesized with the manufacturer's protocol for the Superscript 1st Strand System Kit (Invitrogen). This cDNA was used for PCR analysis on StepOnePlus^{TM} Real-Time PCR System (AppliedBiosystems) with the primers listed in Table 1, and with SYBR Green PCR Master Mix (AppliedBiosystems).

Gene	Forward 5'-3'	Reverse 5'-3'
Col I	GCCAAGAGGAAGGCCAAGTC	AGGGCTCGGGTTTCCACAC
Sox9	GCATGAGCGAGGTGCACTC	TCTCGCTTCAGGTCAGCCTTG
Aggrecan	TGGGAACCAGCCTATACCCCAG	CAGTTGCAGAAGGGCCTTCTGTAC
Runx2	CTTCACAAATCCTCCCCAAGTAGCTACC	GGTTTAGAGTCATCAAGCTTCTGTCTGTG
OPN	GACACATATGATGGCCGAGGTGATAG	GGTGATGTCCTCGTCTGTAGCATC
β-Actin	GCTCCTCCTGAGCGCAAGTAC	GGACTCGTCATACTCCTGCTTGC
Col X	GGAATGCCTGTGTCTGCTTT	TGGGTCATAATGCTGTTGCC
OCN	GTGACGAGTTGGCTGACC	TGGAGAGGAGCAGAACTGG
Col II	CGCCGCTGTCCTTCGGTGTC	AGGGCTCCGGCTTCCACACAT
FABP	ACAGGAAAGTCAAGAGCACCATAA	TGACGCATTCCACCACCAGTT
CEBPA	TCACCGCTCCAATGCCTA	CCTGCTCCCCTCCTTCTCTCA
LEP	TGACACCAAAACCCTCATCAAGAC	GGAGCCCAGGAATGAAGTCCA
LPL	GTCAGAGCCAAAAGAAGCAGC	GGGTTTCACTCTCAGTCCCAG
PPRG	AGGAGAAGCTGTTGGCGG	TGCTTTGGTCAGCGGGAA
DESMIN	CATGAGCCAGGCCTACTCGTC	GCGGGAACACGGGCGAACTCA
MYF5	GCCTCTCAGCAGGATGGA	CTCGCGGCACAAACTCGT

Samples for chondrogenesis of gMSCs: After mRNA extraction, 1 μg of total RNA was reverse- transcribed into cDNA in a 20 μL reaction using random hexamers and the SuperScript First-Strand Synthesis System (Invitrogen). Polymerase chain reaction (PCR) was performed with Taq DNA polymerase (Invitrogen) under following conditions: i) 95 °C for 4 min, ii) 35 cycles of 10 sec denaturation at 95 °C, 30 sec annealing at the primer specific temperature and 1 min at 72 °C for elongation. The reverse transcriptase PCR products were separated by electrophoresis at 100 V on a 2% agarose gel in tris-acetate-EDTA buffer and visualized after staining with ethidium bromide. The primer-sequences were as follows. The sequences of primers (forward and reverse) were as follows:

Gene	Forward 5'-3'	Reverse 5'-3'
Col I	GAGAGACCAAGAACTG	CCATCCAAACCAC-TGAAACC
Sox9	GCATGAGCGAGGTGCACTC	TCTCGCTTCAGGTCAGCCTTG
Aggrecan	CACGCTACACCCTGGACTTG	CCATCTCTCAGGAAGCAGT
Col II	GTGGAGCAGCAAGAGCAAGGA	CTTGCCCCACTTACCAGTGTG
β-Actin	TGGCACCACACCTTCTCAATGAGC	GCACAGCTTCTCCTTAATGTCACGC

Statistical analyses. One-way ANOVA was used to detect significant effects among groups. Tukey's multiple comparison tests were used to detect any significant differences between groups, and a p-value ≤ 0.05 was considered significant. The error bars displayed for the gene-expression data showed the calculated maximum (RQMax) and minimum (RQMin) expression levels that represent the standard deviation of expression level (RQ value). Collectively, the upper and lower limits define the region of expression within which the true expression level value is likely to occur.

Supplementary Figure Legends

Figure S1. **Synthesis and characterization of α-CD-YRGDS/PEGDA hydrogels. a**, α-CD was activated at room temperature with *N*, *N'*-carbonyldiimidazole in DMF followed by its isolation and reaction with YRGDS peptide. **b**, α-CD-YRGDS and PEGDA were mixed

together in PBS (pH 7.4). After overnight mixing, a white precipitate due to threading of α-CD-YRGDS onto PEGDA chains was observed (at higher concentration of α-CD-YRGDS, 10%, w/v). **c**, The hydrogels were synthesized with different concentrations of PEGDA and α-CD-YRGDS. XPS experiment was performed on rigorously washed and dried hydrogels to determine the presence of nitrogen. **d**, The ninhydrin assay was performed on the hydrolyzed hydrogels (at 115 °C in 6 N HCl aqueous solution overnight). The hydrolyzed hydrogel solutions with higher α-CD-YRGDS turned purple compared to the control. The absorbance at 575 nm was correlated to determine the concentration of α-CD-YRGDS in the hydrogels. Data collected throughout this study are presented as a mean ± standard deviation of three or more data samples (*$p \leq 0.05$).

Figure S2. PEGDA 3D hydrogels with functionalized nanobeads – directed differentiation of stem cells. a, The shear storage moduli (~7.0-11.0 kPa) and swelling ratio of the PEGDA hydrogels (10% w/v) with α-CD, α-CD-CH_3, and α-CD-PO_4^- were similar to the control. **b**, The adipogenic differentiation of hADSCs - the presence of hydrophobic group CH_3 increased the relative gene expressions of molecules Leptin (*LEP*) and Peroxisome proliferator-activated receptor gamma (*PPARG*) compared to controls. **c**, The higher concentration of α-CD-OH (5%, w/v) did not change the expressions for adipose-related gene, including *FABP*, *LPL*, *CEBPA*, *LEP*, and *PPARG* compared to 1% α-CD-OH. These expressions for both, 1% and 5% α-CD-OH, except for *LEP*, were similar to the control. **d**, The osteogenic differentiation of hADSCs - no differences in the expressions of osteogenic-related gene (except *COL I*) and alizarin red staining were observed with a low concentration of α-CD-PO_4^- (1%, w/v) compared to 1% (w/v) α-CD-OH and PEGDA. **e**, The enhanced expression of *COL I* is more evident at a higher concentration of α-CD-PO_4^- (5%, w/v), while the relative expression of *RUNX2* remain unchanged. **f**, There were no differences in the expressions of osteogenic-related genes (except *OPN*) for higher concentration of α-CD-OH (5%, w/v) compared to 1% α-CD-OH hydrogel. Data collected throughout the study of compression modulus and swelling ratio are presented as a mean ± standard deviation of three or more data samples.

Supplementary References

[1S] J. B. Mitchell, K. McIntosh, S. Zvonic, S. Garrett, Z. E. Floyd, A. Kloster, Y. D. Halvorsen, R. W. Storms, B. Goh, G. Kilroy, X. Wu, *Stem Cells.* **2006**, *24*, 376.

[2S] C. G. Williams, T. K. Kim, A. Taboas, A. Malik, P. Manson, J. Elisseeff, C. G, *Tiss. Eng*. **2003**, *9*, 679.