Supplementary Materials for

Overexpression of Insulin-Like Growth Factor 1 Enhanced the Osteogenic Capability of Aging Bone Marrow Mesenchymal Stem Cells

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The Supplementary Materials includes:

Table S1A. IGF-1 stimulated DNA synthesis (raw data).

IGF-1 (ng/ml) / Donors	0	1	5	50	500
1	0.255	0.292	0.358	0.381	0.401
2	0.431	0.543	0.637	0.720	0.802
3	0.060	0.075	0.084	0.088	0.105
4	0.042	0.054	0.094	0.082	0.097
5	0.409	0.363	0.399	0.415	0.575
6	0.168	0.167	0.201	0.205	0.228
7	0.523	0.606	0.622	0.618	0.841
8	0.126	0.128	0.139	0.147	0.176
9	0.137	0.150	0.157	0.183	0.312
10	0.136	0.138	0.131	0.133	0.186
11	0.171	0.181	0.161	0.204	0.263
12	0.261	0.259	0.296	0.313	0.362
13	0.039	0.045	0.046	0.050	0.063
14	0.189	0.221	0.219	0.242	0.313

Note. Each value represents the average of OD_{450} readings of 3~4 assays after the subtraction of the readings of PBS background.

Table S1B. IGF-1 stimulated DNA synthesis (donor-specific normalization).

Donors \ IGF-1 (ng/ml)	0	1	5	50	500
1	1	1.1	1.4	1.5	1.6
2	1	1.3	1.5	1.7	1.9
3	1	1.3	1.4	1.5	1.8
4	1	1.3	2.2	2	2.3
5	1	0.9	1	1	1.4
6	1	1	1.2	1.2	1.4
7	1	1.2	1.2	1.2	1.6
8	1	1	1.1	1.2	1.4
9	1	1.1	1.2	1.3	2.3
10	1	1	1	1	1.4
11	1	1	0.9	1.2	1.5
12	1	1	1.1	1.2	1.4
13	1	1	1.2	1.3	1.6
14	1	1.2	1.2	1.3	1.7

Table S2. IGF-1 stimulated alkaline phosphatase expression.

IGF-1 (ng/ml) / Donors	0	50	250
5	1	2.9	3.5
6	1	1.3	1.3
7	1	1.3	1.3
8	1	1.0	1.4
9	1	1.0	2.0
10	1	4.5	1.7
11	1	1.4	1.2
12	1	1.1	1.2
13	1	1.2	1.8
14	1	3.4	1.3

Table S3. IGF-1 stimulated collagen I expression.

IGF-1 (ng/ml) / Donors	0	50	250
5	1	6.2	4.9
6	1	1.3	1.6
7	1	1.2	2.0
8	1	1.4	1.5
9	1	1.4	1.5
10	1	1.3	4.3
11	1	1.4	1.7
12	1	4.4	4.2
13	1	1.1	1.4
14	1	1.1	1.9

Table S4. IGF-1 stimulated osteopontin expression.

IGF-1 (ng/ml) / Donors	0	50	250
5	1	1.1	1.2
6	1	1.2	1.3
7	1	1.3	1.7
8	1	1.0	1.0
9	1	0.9	2.9
10	1	0.9	1.6
11	1	1.2	1.4
12	1	1.2	1.6
13	1	1.1	2.0
14	1	0.9	1.2

Table S5. Characterization of Ca-Alginate scaffolds [32].

(I) Ca^{2+} Concentration of Ca-Alginate Scaffolds	
Weight (mg/scaffold)	***Ca^{2+} (mMol)***
10.1 ± 1.0	0.017 ± 0.006

(II) Porosity of Ca-Alginate Scaffolds	
	Porosity
Ca-Alginate Scaffolds (Freezed-Dry)	~ 96.46 %

(III) Surface Area of Ca-Alginate Scaffolds		
	Sureface Area	***Correlation Coefficient***
(i) BET Surface Area	4.00 ± 0.21 m^2/g	0.998
(ii) Langmuir Surface Area	6.56 ± 0.74 m^2/g	0.994

Characterization of Ca-Alginate scaffolds [32]

The calcium ion concentration was measured by inductively coupled plasma optical emission spectrometry (ICP-OES, Agilent 725, Agilent Technologies). And the porosity of Ca-Alginate scaffolds was determined by mercury intrusion porosimetry (MIP, AutoPore IV 9520, Micyromeritics Instrument). The principle of MIP analysis depends on the intrusion of mercury into porous materials under stringently controlled pressures, and AutoPore IV 9520 provides the dynamic range of pore size distribution between 0.003 to 360 micrometers. Simultaneously, the BET surface area and the Langmuir surface area of Ca-Alginate scaffolds were evaluated by nitrogen adsorption experiments (Micromeritics ASAP 2010 analyzer, Micromeritics Instrument).

[32] Chen CY, Ke CJ, Yen KC, Hsieh HC, Sun JS, Lin FH. 3D porous calcium-alginate scaffolds cell culture system improved human osteoblast cell clusters for cell therapy. Theranostics. 2015; 5: 643-55.

Table S6. Cell numbers of bone-like tissues.

Period of continuous perfusion (days)		Diameters of bone-like tissues (µm)				
			50 µm	100 µm	150 µm	200 µm
Day 7		bmMSC-5	13 ± 1 cells	105 ± 10 cells	355 ± 32 cells	841 ± 76 cells
		bmMSC-5-IGF-1	14 ± 4 cells	115 ± 29 cells	388 ± 98 cells	920 ± 233 cells
Day 14		bmMSC-5	11 ± 1 cells	86 ± 6 cells	290 ± 21 cells	688 ± 50 cells
		bmMSC-5-IGF-1	10 ± 2 cells	81 ± 14 cells	272 ± 48 cells	646 ± 114 cells
Day 21		bmMSC-5	11 ± 0 cells	86 ± 2 cells	290 ± 7 cells	688 ± 18 cells
		bmMSC-5-IGF-1	10 ± 1 cells	82 ± 5 cells	278 ± 17 cells	659 ± 40 cells

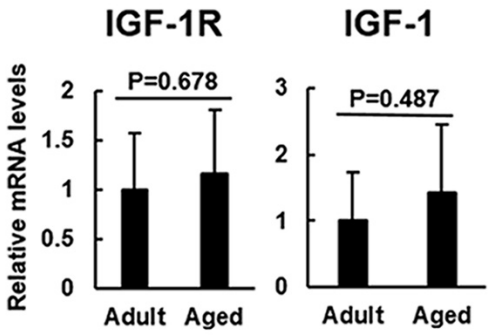

Figure S1. The expression of IGF-1R and IGF-1 in the adult and aged bmMSCs.

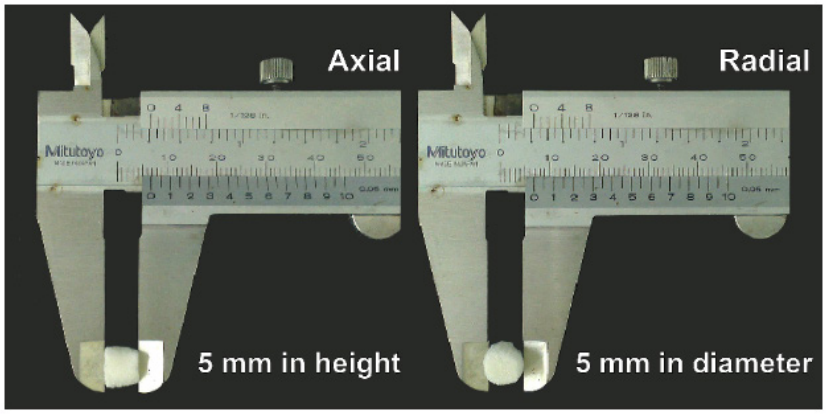

Figure S2. The cylinder-shaped Ca-Alginate scaffolds [32].

Characterization of Ca-Alginate scaffolds and hOBs [32]

The Ca-Alginate scaffolds were fabricated in cylinder shape. We used a vernier caliper to measure the length of the Ca-Alginate scaffolds at axial and radial side.

[32] Chen CY, Ke CJ, Yen KC, Hsieh HC, Sun JS, Lin FH. 3D porous calcium-alginate scaffolds cell culture system improved human osteoblast cell clusters for cell therapy. Theranostics. 2015; 5: 643-55.

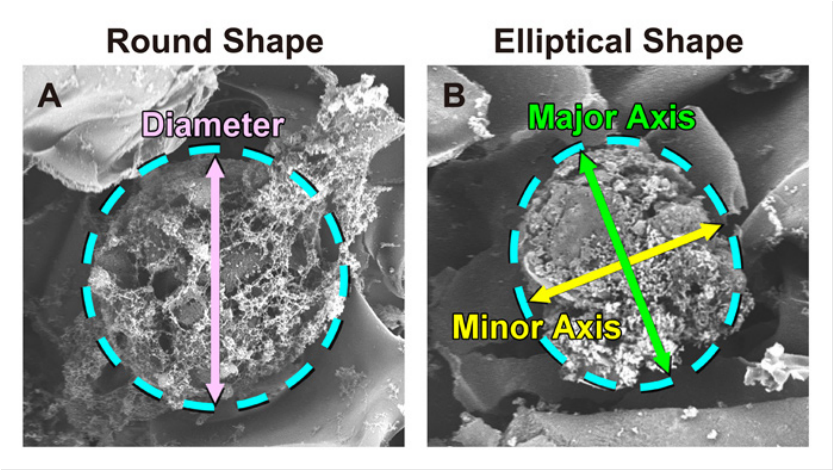

Figure S3. The basis of cell cluster size distribution measurement. (A) we measured the diameter of a round cell cluster; (B) we measured the major axis of an elliptical cell cluster.

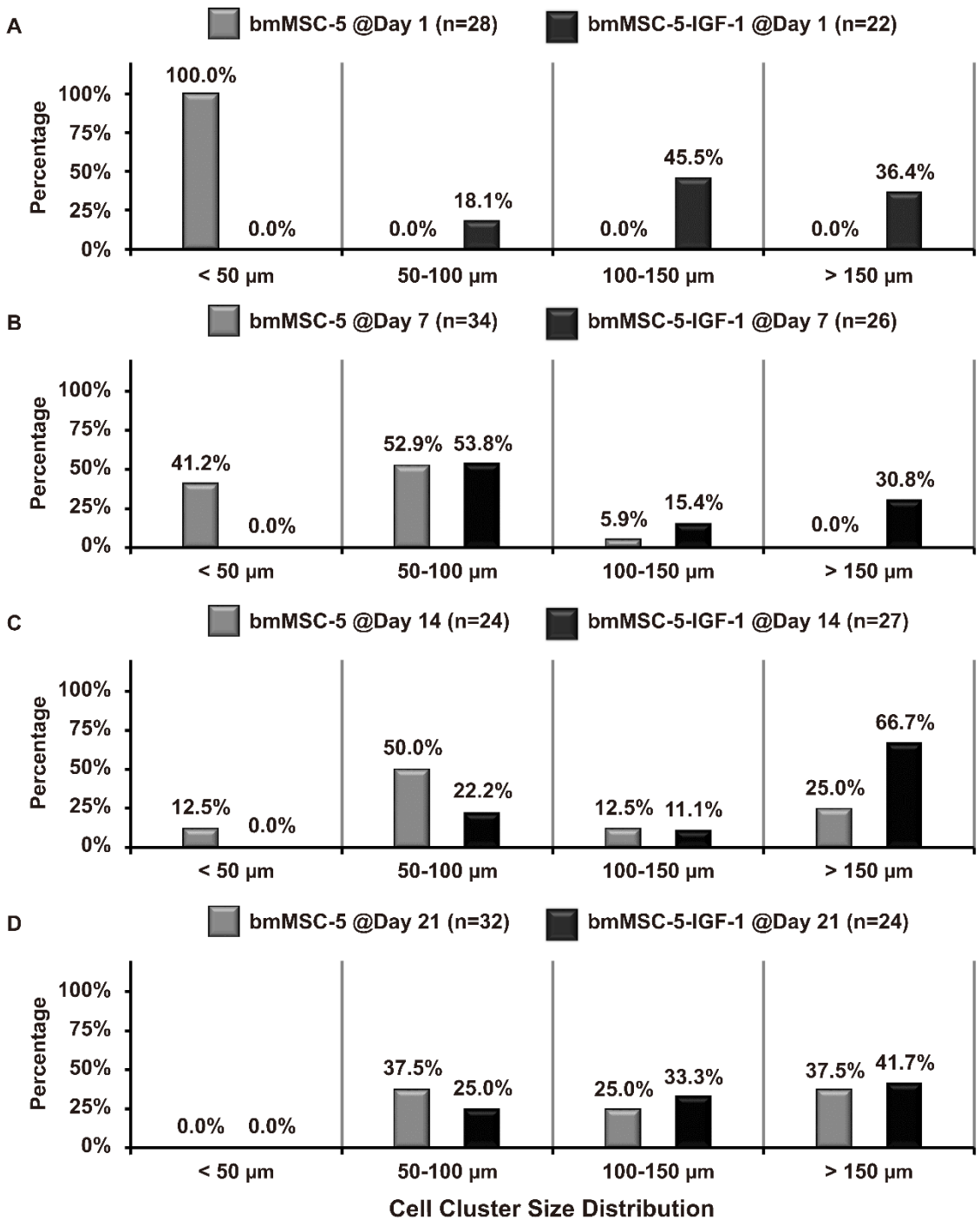

Figure S4. Size distribution of both bmMSC-5 and bmMSC-5-IGF-1 cell clusters under continuous perfusion. (A to D) Both bmMSC-5 and bmMSC-5-IGF-1 cell clusters were examined by SEM under 1000x observation at Day 1 to Day 21 and measured with MetaMorph software.

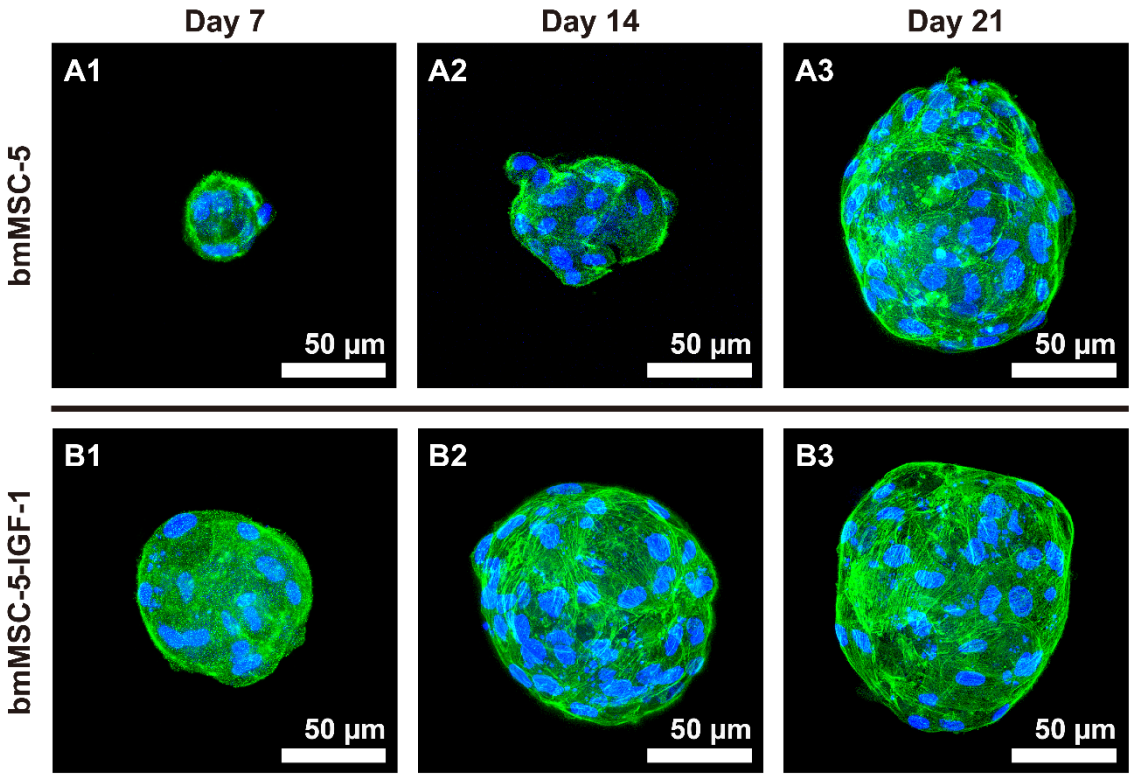

Figure S5 The conformation of both bmMSC-5 and bmMSC-5-IGF-1 cell clusters under continuous perfusion. The data was presented by F-actin staining with green color, and the blue color revealed the nucleus location. (A and B) showed the morphology of both bmMSC-5 and bmMSC-5-IGF-1 cell clusters after 7 to 21 days' perfusion.

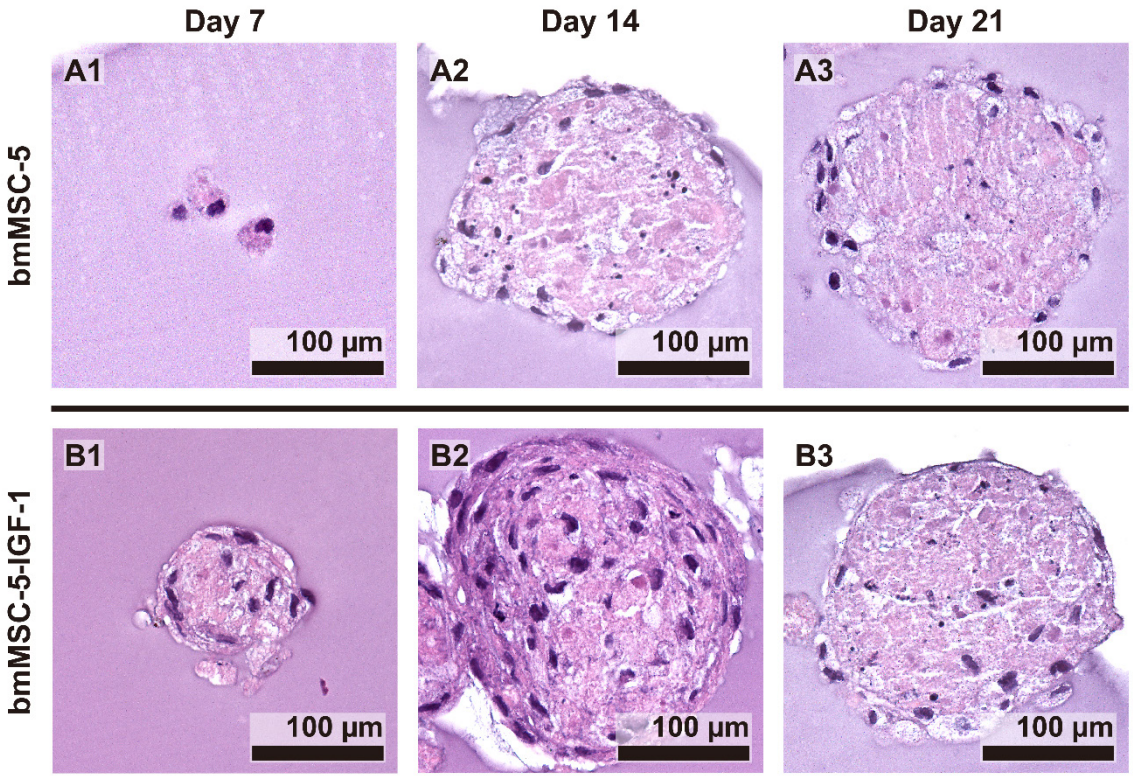

Figure S6. H&E staining of both bmMSC-5 and bmMSC-5-IGF-1 cell clusters under continuous perfusion.