Electronic Supplementary Material (ESI) for Integrative Biology

Supplementary Table 1. Antibodies employed ELISA and immunostaining assessments

Antibody[†]	Clone	Immunostaining	ELISA
Runx2	S-19	√	
Osterix	M-15		√
Alkaline Phosphatase	N-18	√	
Osteopontin	AKm2A1	√	
Osteocalcin	M-15		√
PPARγ	I-18		√
A-FABP	C-15		√
Sox 9	C-20	√	√
Collagen II	N-19		√
Collagen X	E-14	√	√
Myocardin	M-16		√
SM22α	P-15		√
VE-Cadherin	C-19	√	√
PECAM-1	M-20		√
Thrombomodulin	M-17		√
Ki-67	M-19	√	
GAPDH	V-15		√
β-actin	13E5		√

[†] All antibodies were obtained from Santa Cruz Biotechnology, except for β-actin which was obtained from Cell Signaling

Supplementary Table 2. Osteogenic MSC Literature Involving Tensile Conditioning

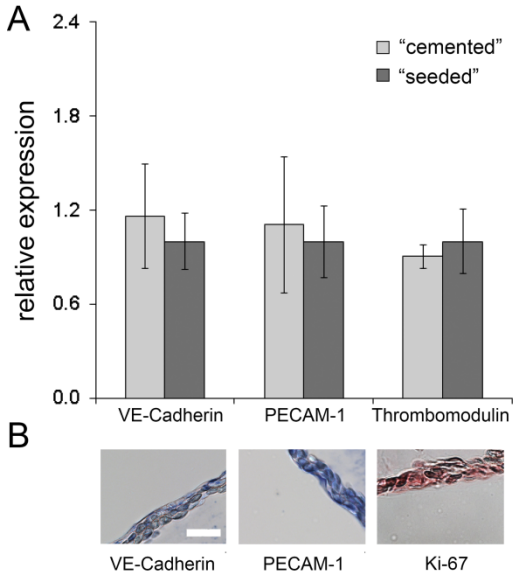
Ref	Cells and Scaffold	Mechanical Conditioning and Media Supplements	Time Points and Analyses	Results
5	hBMSCs, 30,000 cells in 200 µL collagen 3D, collagen gel	*Conditioning*: 1 Hz, 10% or 12% uniaxial tensile strain for 4 h/day *Media Supplements*: No osteoinductive agents	*Time points*: 1, 2 weeks *Assays*: BMP-2 - RT-PCR detection	*10% Strain vs Unloaded* 1 week – **4-fold** increase in BMP-2 compared to the unstrained control 2 weeks – **1.65**-fold increase in BMP-2 *12% Strain vs Unloaded* 1 week – **no** significant differences in BMP-2 2 weeks – **1.75-fold** increase in BMP-2
6	hMSCs, seeded at 10,000 cells/cm^2 2D, collagen coated surfaces	*Conditioning*: 0.25 Hz, 3% equi-biaxial strain (2 s on, 2 s off) *Media Supplements*: 50 mg/ml AA, 10mM BGP, and 0.1 mM DXM	*Time points*: 3, 8, 12, 16 days *Assays*: ALP activity - 50mM p-nitrophenyl phosphate. Mineral deposition - o-cresolphthalein reaction kit	*Loaded vs Unloaded* ALP activity: **no** differences at days 3, 8, 12 or 16 Mineral deposition: loading in a **2.3-fold** increase after 16 days in culture
7	hBMSCs OR 10T½, seeded at 1x10^4 cells/cm^2 2D, flexible silicone rubber BioFlexTM plates coated with collagen I	*Conditioning*: 0.5 Hz, 3% tensile elongation *Media Supplements*: 100 nM DXM, 50 mM AA, and 10 mM BGP	*Time points*: 1, 3, 7, 10 days of loading following 2 days of static culture *Assays*: ALP activity - p-nitrophenyl phosphate (pNPP) Osteogenic genes - RT-PCR. Runx2 protein - western blot.	*hBMSCs - Loaded vs Day 0* Runx2: day 7: **no** increase; day 10: **62%** increase Runx2 protein: day 7: **no** increase; day 10: **60%** increase OP: 7 day: **56%** increase; 10 day: **2-fold** increase. Collagen I: 7 day: **14%** increase; 10 day: **2.3-fold** increase ALP: 7 day: **4.7-fold** increase; 10 day: **6.7-fold** increase *hBMSCs - Loaded vs Unloaded* Runx2: day 7 **60%** decrease; day 10: **56%** decrease Runx2 protein: day 7**: 70%** decrease; day 10: **70%** decrease OP: day 7: **50%** decrease; day 10: **44%** decrease Collagen I: day 7: **53%** decrease; day 10: **40%** decrease ALP: day 7: **~43%** decrease; day 10: **32%** decrease *10T½ Cells - Day 10 – Loaded vs Day 0* Runx2: day 7: **33%** increase; day 10: **78%** increase Runx2 protein: day 7: **3.3-fold** increase; day 10: **5.2-fold** increase OP: day 7: **63%** increase; day 10: **3**-fold increase. Collagen I: day 7: **39%** decrease; day 10: **11%** increase. ALP: day 7: **3-fold** increase; day 10: **5.5-fold** increase *10T½ Cells – Loaded vs Unloaded* Runx2: 7 day: **27%** decrease; 10 day: **38%** decrease OP: 7 day: **50%** decrease; 10 day: **36%** decrease Collagen I: 7 day: **67%** decrease; 10 day: **63%** decrease ALP: 7 day: **85%** decrease; 10 day: **80%** decrease
41	Rat MSCs Silicone strips (10 mm x60 mm) coated with collagen I	*Conditioning*: 0.17 Hz, 2.5% tensile strain *Media Supplements*: No osteoinductive agents	*Time points*: 3, 6, 9, 14 *Assays*: Runx2, Collagen I, and OCN - Immunocytochemistry BMP2 - western blot	*Loaded vs Unloaded* Runx2: **2-fold** increase at day 6 Collagen I: **1.76-fold** increase at day 6 OCN: **1.5-fold** increase at day 6 BMP2: **4.8-fold** increase at day 14

Abbreviations: TGF-β1 Transforming growth factor β1, AA – ascorbic acid, DXM – dexamethasone, BGP- β-glycerophosphate , PG – proteoglycan, ALP – alkaline phosphatase, OP – osteopontin, OCN – osteocalcin, BSP – bone sialoprotein; HUVEC – human umbilical vein endothelial cells; MSC –mesenchymal stem cells; hBMSCs – human bone marrow-derived MSCs

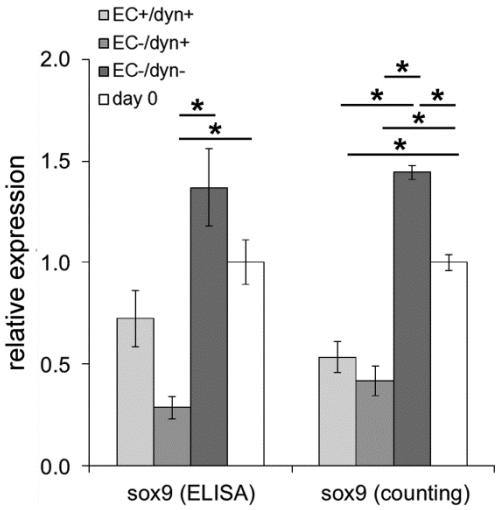
Supplementary Table 3. Endothelial Cell – MSC Co-Culture Literature

Ref	Cells and Scaffold	Mechanical Conditioning and Media Supplements	Time Points and Analyses	Results
8	EC and BMSC,1x10^4 cells/cm^2 2D study: direct and indirect contact	*Conditioning*: Static *Media Supplements*: 50:50 mixture of EC culture medium (EGM2-MV) and BMSC culture medium (α-MEM, 10% FBS)	*Time points*: 3, 14 days *Assays*: ALP activity - colorimetric assay OCN – ELISA	*Direct Contact EC-MSC Co-Culture vs Mono-Culture (1:1 ratio)* ALP: at 2 wks, a **2-fold** increase was observed in co-cultured BMSCs OCN: a **1.4-fold** increase in OCN expression in co-cultured BMSCs *Indirect Contact* **No** significant differences - data not shown
9	hMSC and HUVEC 15,000 cells each per spheroid (30,000 cells per spheroid) 3D study	*Conditioning*: Static *Media Supplements*: 5 mM BGP, 50 μg/mL AA, and 10 nM DXM	*Time points*: 7, 14 days *Assays*: ALP levels - fluorescence images	*MSCs and ECs Directly Co-cultured at a 1:1 Ratio* ALP levels: At day 7, ~**5 times** higher in MSC/HUVECs than in HDFa/MSC controls. At day 14, ~**26 times** higher in MSC/HUVECs than in HDFa/MSC controls
10	HUVEC and hMSC $5x10^5$ cells/spheroid 3D study	*Conditioning:* Static *Media Supplements*: 10% FBS, 0.2mM AA, 2 mM L-glutamine, 10 nM DXM, and 0.01M BGP	*Time points*: 10 days *Assays*: ALP - RT-PCR	*EC-hMSC Spheroids with 100% hMSCs or 95% hMSCs Plus 5% HUVECs* ALP: **4-fold** increase (plus or minus 1.7) by adding 5% HUVECs to the spheroids
42	MSC and HUVEC $3x10^3$ cells/cm^2 2D co-culture: direct contact	*Conditioning*: Static *Media Supplements*: Basal: M199 +DMEM Osteogenic media: DMEM+ M199 with 10 mM BGP, 10 nM DXM, 50 μg/mL AA	*Time points*: 1, 2, 3 weeks *Assays*: Osteogenic genes - RT-PCR ALP activity - 2 mM ρ–nitrophenol phosphate	*MSC-EC Co-Culture vs Mono-culture* ALP: **50%** higher levels in co-culture Collagen I: **2.2-fold** higher levels in co-culture BSP: **7.5-fold** increase in co-culture at 1 week Runx2: **50%** increase in co-culture at 1 week OCN: **no** significant differences *MSC-EC Co-Culture vs Day 0* ALP: day 7: **2-fold** increase; day 14: **3.8-fold** increase; day 21: **4-fold** increase. Collagen I: day 7: **1.3-fold** increase; day 14: **1.4 fold** increase; day 21: **1.7-fold** increase BSP: day 7: **11.5-fold** increase *Different EC-MSC Cell Ratios:* Basal conditions: ALP activity increased in co-co-cultures after 3 wks. 25%EC-75%MSC: **29-fold**; 50%EC-50%MSC: **18-fold** Osteogenic conditions: ALP activity increased in 3 wk co-cultures: 25%EC-75%MSC: **3.5 fold**; 50%EC-50%MSC: **3.7 fold** 25%EC-75%MSC

Abbreviations: TGF-β1 Transforming growth factor β1, AA – ascorbic acid, DXM – dexamethasone, BGP- β-glycerophosphate , PG – proteoglycan, ALP – alkaline phosphatase, OP – osteopontin, OCN – osteocalcin, BSP – bone sialoprotein; HUVEC – human umbilical vein endothelial cells; MSC –mesenchymal stem cell; hMSC – human MSCs



Supplementary Figure 1. (A) The phenotype of "cemented" ECs was evaluated relative to conventionally "seeded" ECs, i.e. "cemented" ECs were compared to ECs which had been seeded as a confluent monolayer on the surface of PEGDA hydrogels containing cell adhesion ligand RGDS. Competitive ELISAs indicated that ECs in both contexts expressed similar levels of VE-Cadherin and PECAM-1, markers associated with EC intercellular junctions and permeability regulation. Furthermore, competitive ELISAs were conducted for thrombomodulin, a protein downregulated following EC damage.[43] As for VE-Cadherin and PECAM-1, levels of thrombomodulin were similar for both "cemented" and conventionally "seeded" ECs. These results are consistent with a previous study demonstrating that "cemented" ECs display a similar phenotype as conventionally "seeded" ECs.[16] (B) "Cemented" EC layers immunostained for VE-Cadherin, PECAM-1, and Ki-67 (a nuclear protein associated with cell proliferation). Scale bar = 40 μm.



Supplementary Figure 2. Comparison of day 22 sox9 levels as assessed by ELISA and cell counting techniques. For ELISA assays, 3-4 samples per treatment group were analyzed. For cell counts, sections from at least 3 separate samples of each treatment group were evaluated. The degree of correlation between the two assessment techniques was 99.2% by Pearson's correlation coefficient method.