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Appendix

Fig. E-1
Morphological evaluation of human bone-marrow-cell fractions. The fractions of human bone marrow cells within the hTEBOs without rhBMP-7 (**Figs. E-1A and E-1B**) and with rhBMP-7 (**Figs. E-1C and E-1D**) were analyzed after 10 weeks of in vivo growth. Hematoxylin and eosin (H & E) staining of the hTEBO with rhBMP-7 (**Figs. E-1C and E-1D**) and the mouse femur (**Figs. E-1E and E-1F**) suggested equal bone-marrow-cell morphology, whereas hTEBOs growing without rhBMP-7 were filled only with fibrous and adipose tissue (**Figs. E-1A and E-1B**). Flow cytometric analysis was performed in 2 hTEBOs without rhBMP-7 (**Fig. E-1G [i, ii, and iii]**) and 2 with rhBMP-7 (**Fig. E-1H [i, ii, and iii]**). HuCD45+ leukocytic cells (**Figs. E-1G [i] and E-1H [i]**) and huCD34+ HSCs (**Figs. E-1G [ii] and E-1H [ii]**) were only present in the rhBMP-7 ossicles (**Figs. E-1H [i] and E-1H [ii]**). HuCD3+ T cells and huCD19+/CD20+ B cells as well as huCD14+ monocytes also were only detected within the hTEBOs with added rhBMP-7 (**Figs. E-1H [ii] and E-1H [iii]**). The graphs show results of representative hTEBOs. Tr = trabeculae, Cx = cortex, BM = bone marrow, GP = growth plate, and FT = fibrous tissue.

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TABLE E-1 Utilized Antibodies and Experimental Conditions for Immunohistochemical Staining*

Antibody	Antigen Retrieval Method	Dilution	Stained Structure	Source
hsNuMa	Sodium citrate buffer, pH 6.0 (4 min, 95°C)	1:100	Detects all human cell types	Abcam (ab97585)
hsCol-I	Sodium citrate buffer, pH 6.0 (4 min, 95°C)	1:300	hsCol-I	MP Biomedicals (I-8H5)
hsOsteocalcin	Proteinase K (25 min, RT)	1:300	Human osteocalcin (not reactive to mouse)	Abcam (ab13418)
hsCD146	Tris-EDTA buffer, pH 9.0 (10 min, 95°C)	1:25	Human perivascular mesenchymal progenitor cells	Leica Biosystems (NCL-CD146)
hsCD45	Sodium citrate buffer, pH 6.0 (4 min, 95°C)	1:100	Leukocyte common antigen	Dako (M0701)
VEGF	DAKO AR solution (high pH) (20 min, 95°C)	1:400	VEGF	Santa Cruz Biotechnology (Sc-152)
HIF-1α	DAKO AR solution (high pH) (20 min, 95°C)	1:300	HIF-1α	Novus Biologicals (NB100-105)
HIF-2α	Proteinase K (20 min, RT)	1:400	HIF-2α	Novus Biologicals (NB100-122)
Ki67	Tris EDTA buffer, pH 9.0 (10 min, 95°C)	1:75	Proliferating cells	Dako (M7240)
Periostin	Sodium citrate buffer, pH 6.0 (2 min, 95°C)	1:50	Extracellular matrix component expressed by fibroblasts	Novus Biologicals (NBP1-30042)

*Hs = human specific and RT = room temperature.