

Simple Establishment of a Vascularized Osteogenic Bone Marrow Niche Using Pre-Cast Poly(ethylene Glycol) (PEG) Hydrogels in an Imaging Microplate

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Materials

Name	Company	Catalog Number	Comments
0.25% Trypsin-EDTA	Gibco	25200-072	
2 mL microtubes	Eppendorf	30120094	
2-Amino-2-methyl-1-propanol	Sigma	A9199	
3DProSeed hydrogel well plate	Ectica Technologies	ECT.PS1.001.096	
4-Nitrophenyl phosphate disodium salt hexahydrate	Sigma	71768	
Alizarin Red S	Sigma	A5533	
Anti-Collagen IV antibody	Abcam	ab6311	
Anti-Laminin 1+2 antibody	Abcam	ab7463	
Automated plate washer	Agilent Biotek	ELx50	
Automated washer/dispenser	Agilent Biotek	MULTIFLO FX equipped with a peristaltic pump 5uL cassette	
Bevacizumab	Evidentic	ID PS-E07-2019-00119 A009	
BMP-2	Peprtech	120-02C	
BSA	AppliChem	A1391	
Centrifuge	Eppendorf	5415 R	To centrifuge 2 mL tubes at 16100 x g during ALP analysis
Confocal laser scanning microscope	Leica	Stellaris 5	
Conical 50 mL centrifuge tubes	TPP	91050	
DAPI	Sigma	D9542	
DyLight 649 Donkey anti-rabbit IgG (minimal x-reactivity) Antibody	Biologend	406406	
DyLight 649 Goat anti-mouse IgG (minimal x-reactivity) Antibody	Biologend	405312	
EGM-2	Lonza	CC-3162	
Epifluorescence microscope	Leica	DMI6000B	
FBS	Gibco	10500-064	

FGF-2	Peprotech	100-18B	
Fibronectin (IST-9)	Santa Cruz	sc-59826	
GFP-HUVECs	PELOBiotech	PB-CAP-0001GFP	
hBM-MSCs	-	-	Isolated at University Hospital Basel; Papadimitropoulos A, Piccinini E, Brachat S, et al. Expansion of human mesenchymal stromal cells from fresh bone marrow in a 3D scaffold-based system under direct perfusion. PLoS One. 2014;9(7):e102359
Inverted microscope	Zeiss	200M	
Magnesium chloride	Sigma	M8266	
MDA-MB-231 breast cancer cell line	-		Kindly obtained from J Massagué at the Memorial Sloan-Kettering Cancer Center
MEM α	Gibco	22571-038	
Multimode imaging reader	Agilent Biotek	Cytation 1	For automated imaging
Multimode imaging reader - fluorescence and absorbance	Agilent Biotek	Cytation 5	For measuring absorbance and fluorescence intensity during ALP analysis
Paraformaldehyde	Artechemis	US 040	
PBS	Gibco	10010-015	
Penicillin/Streptomycin	Gibco	15140-122	
Phalloidin-rhodamine	Invitrogen	R415	
Picro-Sirius Red Solution	Abcam	ab246832	
Quant-iT PicoGreen dsDNA Assay kit	ThermoFisher Scientific	P7589	
Recombinant Anti-Collagen I antibody	Abcam	ab260043	
SIGMAFAST BCIP/NBT	Sigma	B5655-25TAB	
Sodium hydroxide	Sigma	1064981000	
Sodium phosphate dibasic, anhydrous	Sigma	S-0876	
Sodium phosphate monobasic, monohydrate	Merck	1.06346	
Triton X-100	Sigma	T8787	
Tween20	AppliChem	A4974	
U2OS osteosarcoma cell line	-		Kindly obtained from J Snedeker at the Institute for Biomechanics, Zurich
α -trehalose dihydrate	Sigma	90208	