Supplementary Documents

Chitosan-Collagen 3D Matrix Mimics Trabecular Bone and Regulates RANKL-Mediated Paracrine Cues of Differentiated Osteoblast and Mesenchymal Stem Cells for Bone Marrow Macrophages-Derived Osteoclastogenesis

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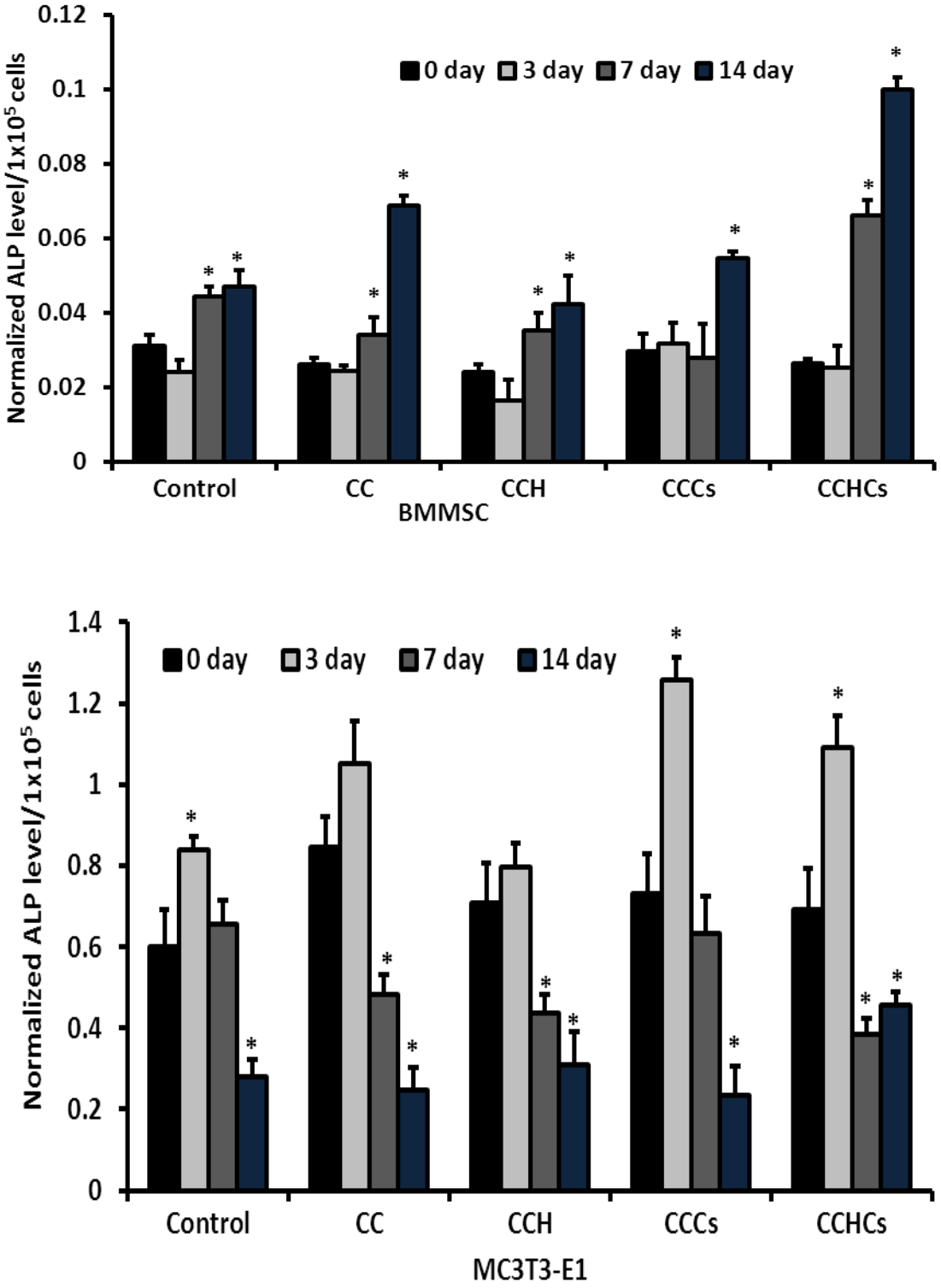
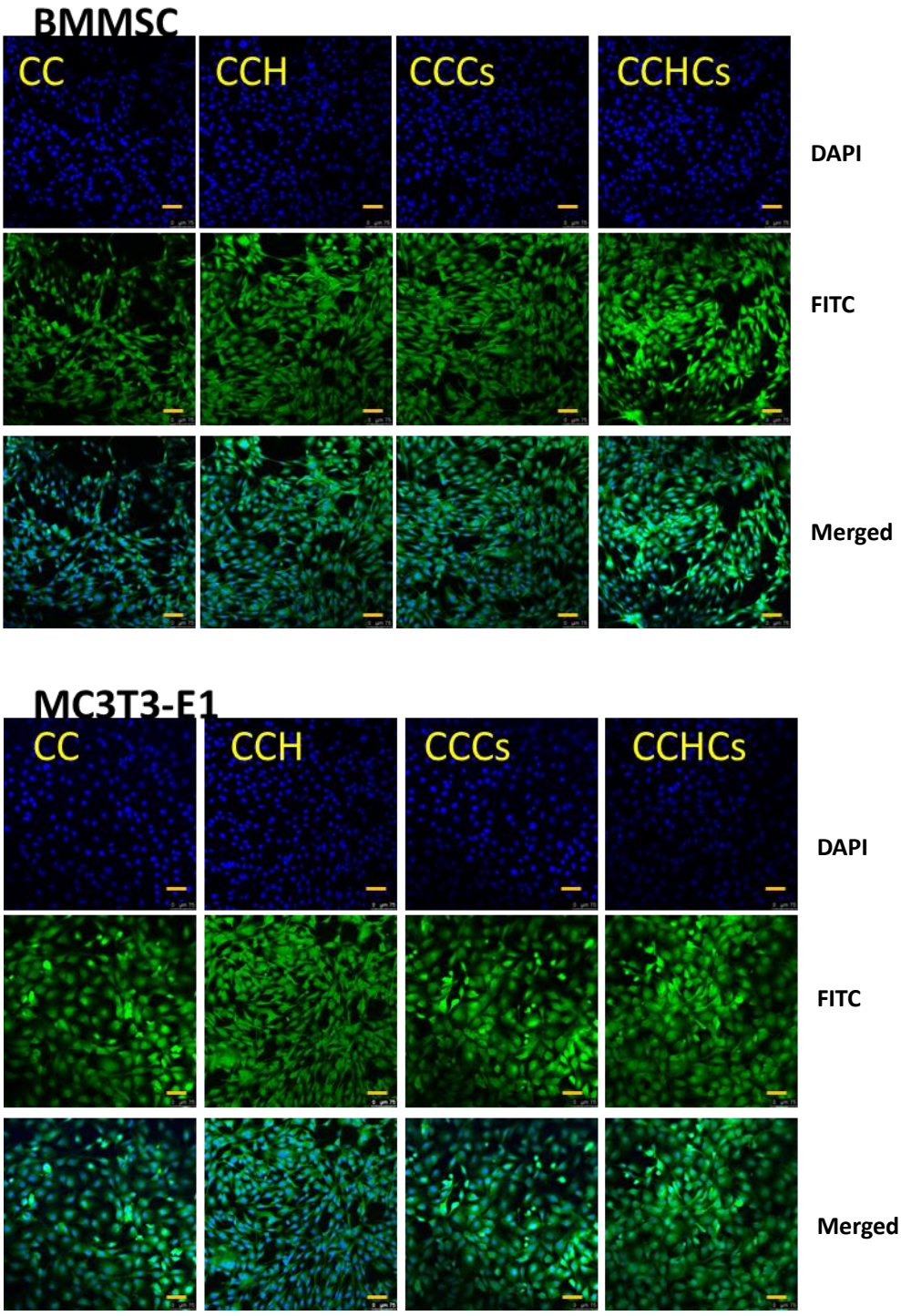
Supplementary Table S1. List of Primers and its sequence used in this study

S.No	Primers	Sequences
1	GAPDH	5'- AGC TTG TCA TCA ACG GGA AG-3'
		5'- TTT GAT GTT AGT GGG GTC TCG-3'
2	COL I	5'- GCG AAG GCA ACA GTC GCT-3'
		5'- CTT GGT GGT TTT GTA TTC GAT GAC-3'
3	Osteocalcin (OC)	5'- CTC ACA GAT GCC AAG CCC-3'
		5'- CCA AGG TAG CGC CGG AGT CT-3'
4	Alkaline Phosphatase (ALP)	5'- TCC TGA CCA AAA ACC TCA AAG G-3'
		5'- TGC TTC ATG CAG AGC CTG C-3'
5	RUNX2	5'- CCA CCA CTC ACT ACC ACA CG-3'
		5'- TCA GCG TCA ACA CCA TCA TT-3'
6	RANKL	5'- CCT GAG GCC CAG CCA TTT-3'
		5'- CTT GGC CCA GCC TCG AT-3'



Supplementary Fig S1. The level of ALP in bone cells. ALP level was normalized with corresponding cell numbers at each time point. BMMSC: Bone Marrow-derived Mesenchymal Stem Cell, MC3T3-E1:pre-osteoblast. CC: collagen-chitosan 3D matrix, CCH: collagen-chitosan-hydroxyapatite 3D matrix, CCCs: collagen-chitosan-chondroitin sulphate 3D matrix and CCHCs: collagen-chitosan-hydroxyapatite-chondroitin sulphate 3D matrix.*$p < 0.05$ vs 0 day.



Supplementary Fig S2. Confocal laser scanning microscope (CLSM) images of bone cells cultured on 3D matrix conditioned medium (scale bars: 75 μm). For confocal microscopy, bone cells were grown in a matrix conditioned medium with osteogenic stimulatory medium for 14 days and stained with FITC and DAPI. BMMSC: Bone Marrow-derived Mesenchymal Stem Cell, MC3T3-E1:pre-osteoblast. CC: Collagen-chitosan 3D matrix, CCH: collagen-chitosan-hydroxyapatite 3D matrix, CCCS: collagen-chitosan-chondroitin sulphate 3D matrix and CCHCS: collagen-chitosan-hydroxyapatite-chondroitin sulphate 3D matrix.