pathogens

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Article

3D Cocultures of Osteoblasts and *Staphylococcus aureus* on Biomimetic Bone Scaffolds as a Tool to Investigate the Host–Pathogen Interface in Osteomyelitis

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Supplementary Materials and Methods

Sequences of the primers used in the RT-PCR esperiments are listed in Table S1 (for the MC3T3-E1 murine cells) and Table S2 (for the Newmann strain of *Staphylococcus aureus* used in the study).

Table S1. RT-PCR murine primers.

Targeted Gene (mouse)	Primer Sequences (5′-3′)
18S	F: CTCAACACGGGAAACCTCAC R: CGCTCCAACTAAGAAGG
Ptx3	F: CGAAATAGACAATGGACTCCATCC R: CAGGCGCACGGCGT
Spp1	F: GAAGGCTCATGGTTGGATGT R: GTAGCCCAAGGGTATTTCAG
Col1a1	F: CTCCTGGTATTGCTGGTGCT R: TTCACCAGGAGAACCTTTGG
NRf2	F: CATGATGGACTTGGAGTTGC R: CCTCCAAAGGATGTCAATCAA
Ho-1	F: AGGCTAAGACCGCCTTCCT R: TGTGTTCCTCTGTCAGCATCA
Alp	F: AAGGCTTCTTCTTGCTGGTG R: GGTGTATCCACCGAATGTGA
Tgf-β	F: GTCAGCAGCAGCCGGTTACCA R: TGGAGCAACATGTGGAACTC
Opg	F: GTTCCCGAGGACCACAAT R: CCATTCAATGATGTCCAGGAG
Tnf-α	F: GACGTGGAACTGGCAGAAGAG R: TTGGTGGTTTGTGAGTGTGAG
Bmp2	F: CGGACTGCGGTCTCCTAA R: GGGGAAGCAGCAACACTAGA
Zo-1	F: ATGCAGACCCAGCAAAGGT R: TGACCAAGAGCTGGTTGTTTT

Table S2. RT-PCR *Staphylococcus aureus* primers.

Targeted Gene (*Staphylococcus Aureus*)	Primer Sequences (5′-3′)
16S	F: GCCACACTGGAACTGAGACA R: AGTTAGCCGTGGCTTTCTGA
Spa	F: AAGAAGACGGCACGGAGTA R: TTAGCATCTGCATGGTTTGC
Psm	F: TGTCATACCCCAGCAGAGTG R: TAATGGCGCTTGGCTTTATT
ClfA	F: CAACTGCTAAAGTGCCACCA R: GTCAATATAAGCGGGCATGG
agrRNAIII	F: AAAGTTGCAGCGATGGATTT R: AAATGCGCAATGAGTCTGTG
FnbpA	F: CCAGGTGGTGGTCAGGTTAC R: GTGCTTGACCATGCTCTTCA

Supplementary Figures

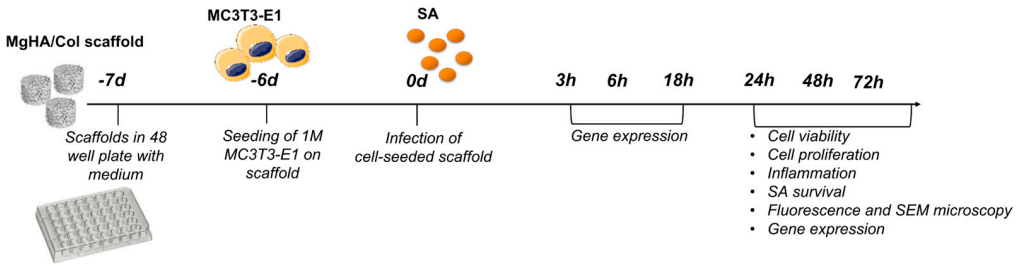

Figure S1. Experimental design and outcomes of the *in vitro* model of OM developed in the study (MC3T3-E1/SA co-cultures on MgHA/Col bone biomimetic scaffolds).

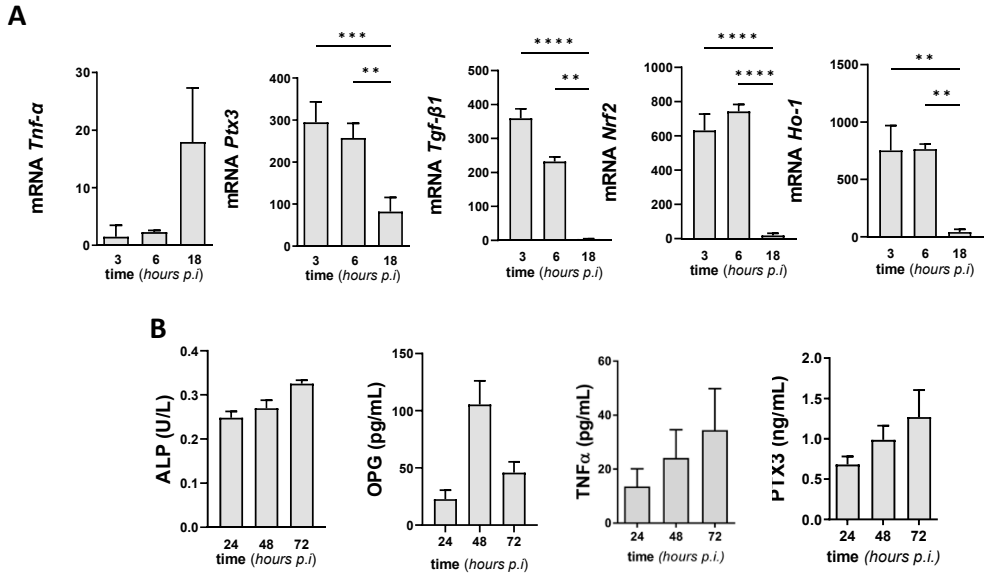

Figure S2. Gene and protein expression of SA-infected MC3T3-E1 cells in 2D co-cultures. MC3T3-E1 cells were seeded on MgHA/Col 3D scaffolds, and cultured for 6 days. Live SA was then inoculated (MOI of 160:1), and co-cultured with MC3T3-E1 cells up to 72 hours. (**A**) Cells were harvested at the reported time points, total RNA was extracted, and mRNA levels of the indicated genes were measured by qRT-PCR. Data were normalized based on the levels of 18S rRNA, and expressed as mean ± SEM (n = 4–6 from 2 to 3 independent experiments preformed in duplicate). (**B**) Activity of ALP and concentration of OPG, TNF-α, and PTX3 in the conditioned medium of MC3T3-E1/SA co-cultures were determined using commercial enzymatic and ELISA kits. Data are from 3–4 independent experiments performed in duplicate or triplicate (n = 7, mean ± SEM). In all panels, **** $p < 0.001$, *** $p < 0.005$, ** $p < 0.01$, * $p < 0.05$, one-way ANOVA test with Dunn's multiple comparisons test.