applied sciences

MDPI

SUPPLEMENT

Anti-inflammatory activity of a demineralized bone matrix: An in vitro pilot study

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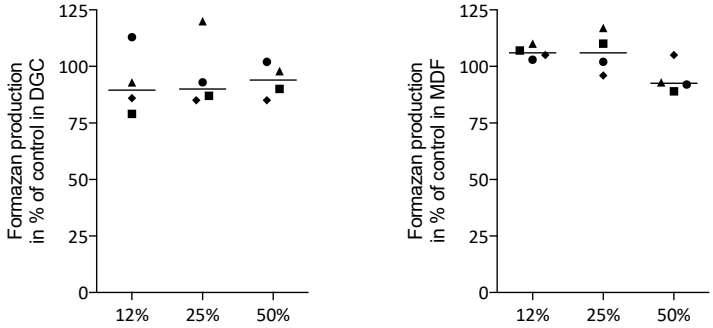
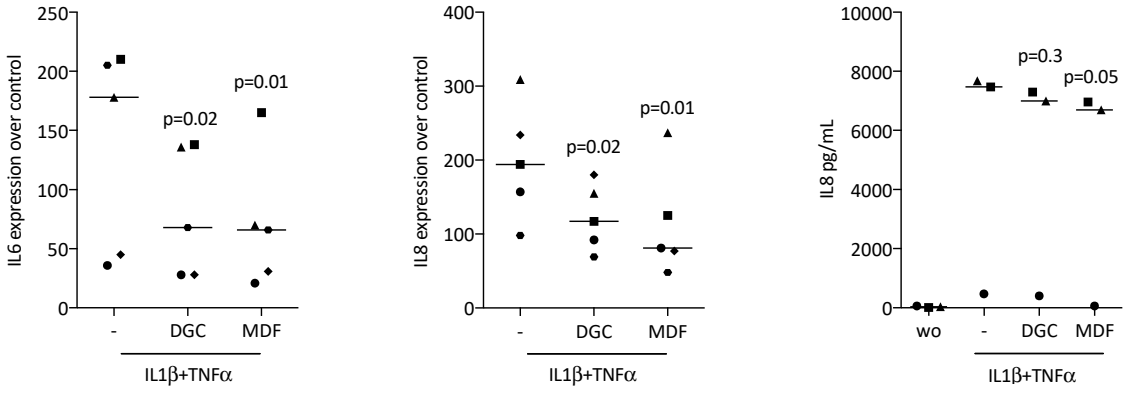


Supplement Figure S1: DGC (left) and MDF (right) maintain the viability of RAW 264.7 macrophages. The RAW 264.7 cells were grown in the presence of 12, 25 and 50% allograft lysates for 24 hours followed by a classical MTT assay. As indicated in Supplementary Figure 1, there was no considerable change in cell viability.



Supplement Figure S2: DGC and MDF moderately reduce the expression of IL6 and IL8 in IL1β and TNFα-stimulated gingival fibroblasts. Gingival fibroblasts were exposed to 25% acid lysates of DGC and MDF for 30 minutes followed by 10 ng/ml IL1β and TNFα for 24 hours. IL8 ELISA was done with the respective supernatant. Data show the relative expression changes normalized to the untreated cells. The experiments were performed five times, represented by a unique symbol for each replicate. Statistical analysis is based on a Friedmann test and p-values are indicated.

Supplement Table S1: Primer sequences of proliferation marker genes. RAW 264.7 macrophages were exposed to DGC lysates and gene expression analyzed; there we no obvious changes in the expression of the proliferation marker genes (data not shown).

hCCND1	TCGGTGTCCTACTTCAAATGTGT	GGGATGGTCTCCTTCATCTTAG
hKi-67	ATAAACACCCCAACACACACAA	GCCACTTCTTCATTCCAGTTACA
hPCNA	CCTGCTGGGATATTAGCTCCA	CAGCGGTAGGTGTCGAAGC

Supplement Table S2: Blocking of the TGF-β receptor type I kinase with SB431542 failed to reverse the anti-inflammatory activity of the DGC lysate. The data show the expression changes of IL1 and IL6 of RAW 264.7 macrophages exposed to LPS alone or the combination with DGC and DGC with SB431542. SB431542 failed to reverse the inhibitory effect of DGC suggesting that the activity of DGC is not caused by modulation of TGF-β signaling.

	LPS	LPS + DGC	LPS + DGC + SB431542
Experiment 1 (IL1/IL6)	434.4/914.0	32.0/170.1	42.2/211.3
Experiment 2 (IL1/IL6)	2068.0/1163.0	957.5/294.2	948.9/176.9