CHAPTER 3

RESULTS

3.1 Effects of EMD on the ratio of RANKL/OPG mRNA levels in osteoblast-like cell MG-63

The effects of EMD on OPG and RANKL mRNA levels in osteoblast-like cell MG-63 over time course was determined by semiquantitative RT-PCR assay. The chronology of osteoblast-like cells MG63 responsed to EMD 100 μg/ml on the expression of OPG (Fig.3.1) and RANKL mRNA levels (Fig.3.2) was examined at 0, 6, 12, 24 and 48 hours of cultures. The cultures with free FBS and 10% FBS were employed as controls. The values for treated and untreated (controls) samples were corrected for any variations due to differences in lane loading by standardization for the levels of GAPDH mRNA Compared to controls, treatment with 100 μg/ml EMD resulted in no change in mRNA level of OPG at 0 h. However, with longer treatment, there was a trend of the decrease in the level of OPG mRNA of which the results were first detected at 6 h of cultures. Similar results were found in the determination of RANKL mRNA expression in the EMD treated cultures compared with controls. There were no significantly differences in the level of OPG and RANKL mRNAs among EMD-treated cultures and controls over various time points as indicated.

Based on above findings, the calculated RANKL/OPG mRNA ratio was found no change over various time points as indicated (Fig.3.3). In addition, the comparison ratio of mRNA expression of RANKL/OPG among EMD treated cultures and free FSB and 10% FSB over various times of cultures was found no significantly difference. However, the ratio of RANKL/OPG mRNA level of 48 h of EMD treated cultures seemed to be higher than the controls.

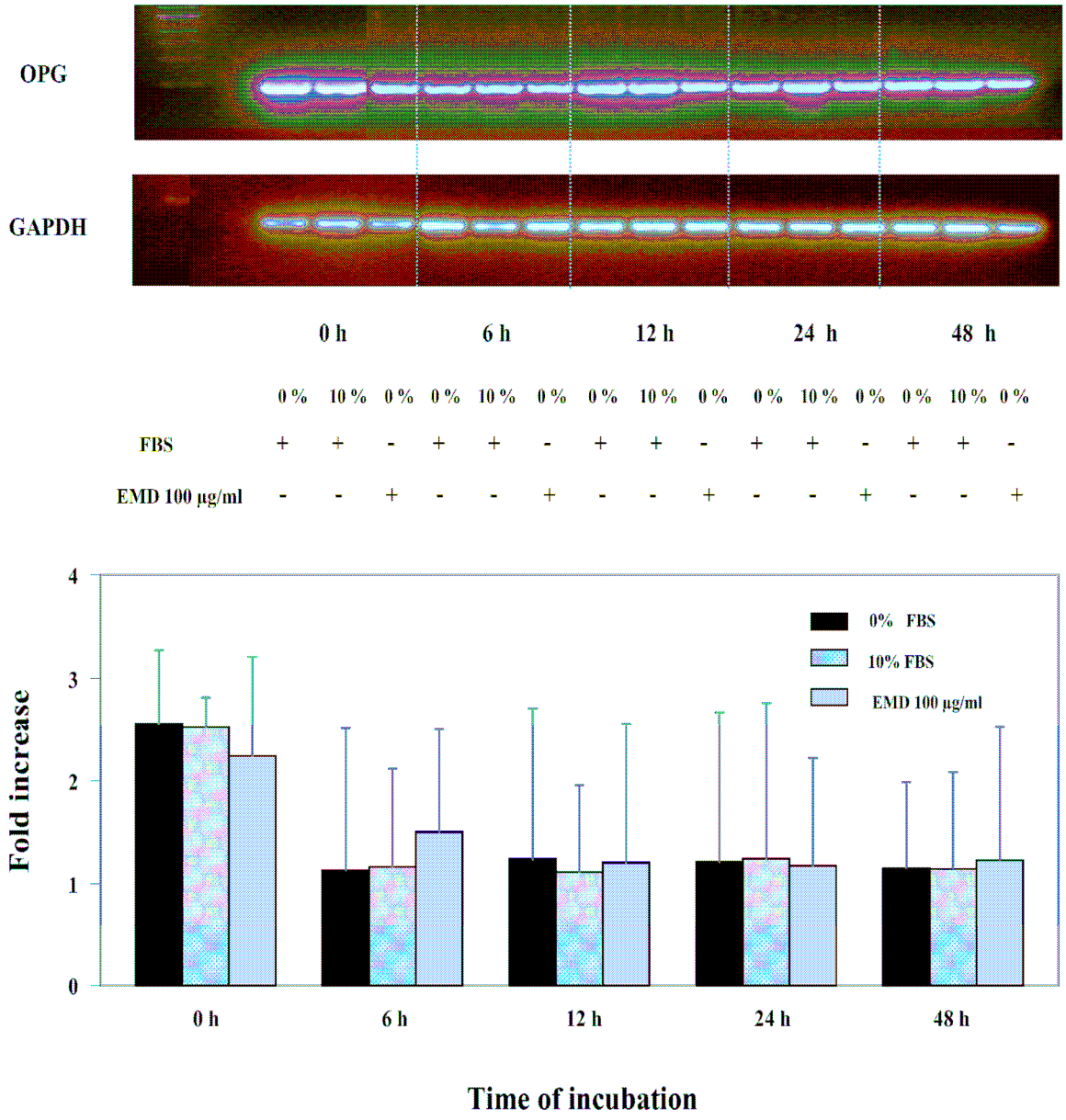


Figure 3.1 Graphical representation of OPG mRNA expression in osteoblasts-like cell MG-63 treated with 0% FBS, 10% FBS and EMD 100 µg/ml for 0, 6, 12, 24 and 48 h, amplified by semiquantitative RT-PCR. Each value represents mean ± SD of duplicate cultures. The figure shows representative data from one of duplicate experiments.

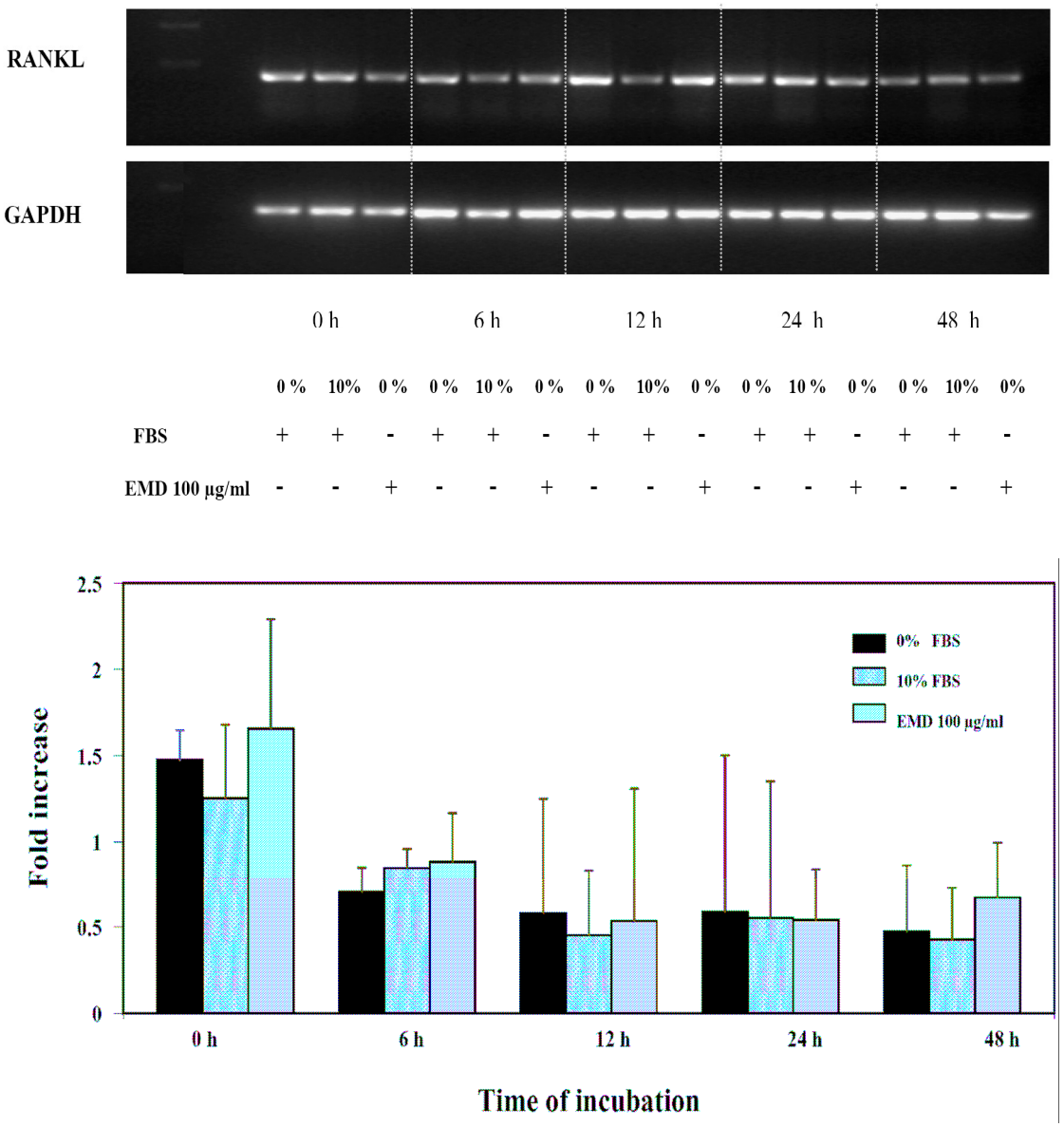


Figure 3.2 Graphical representation of RANKL mRNA expression in osteoblasts-like cell MG-63 treated with 0% FBS, 10% FBS and EMD 100 µg/ml for 0, 6, 12, 24 and 48 h, amplified by semiquantitative RT-PCR. Each value represents mean ± SD of duplicate cultures. The figure shows representative data from one of duplicate experiments.

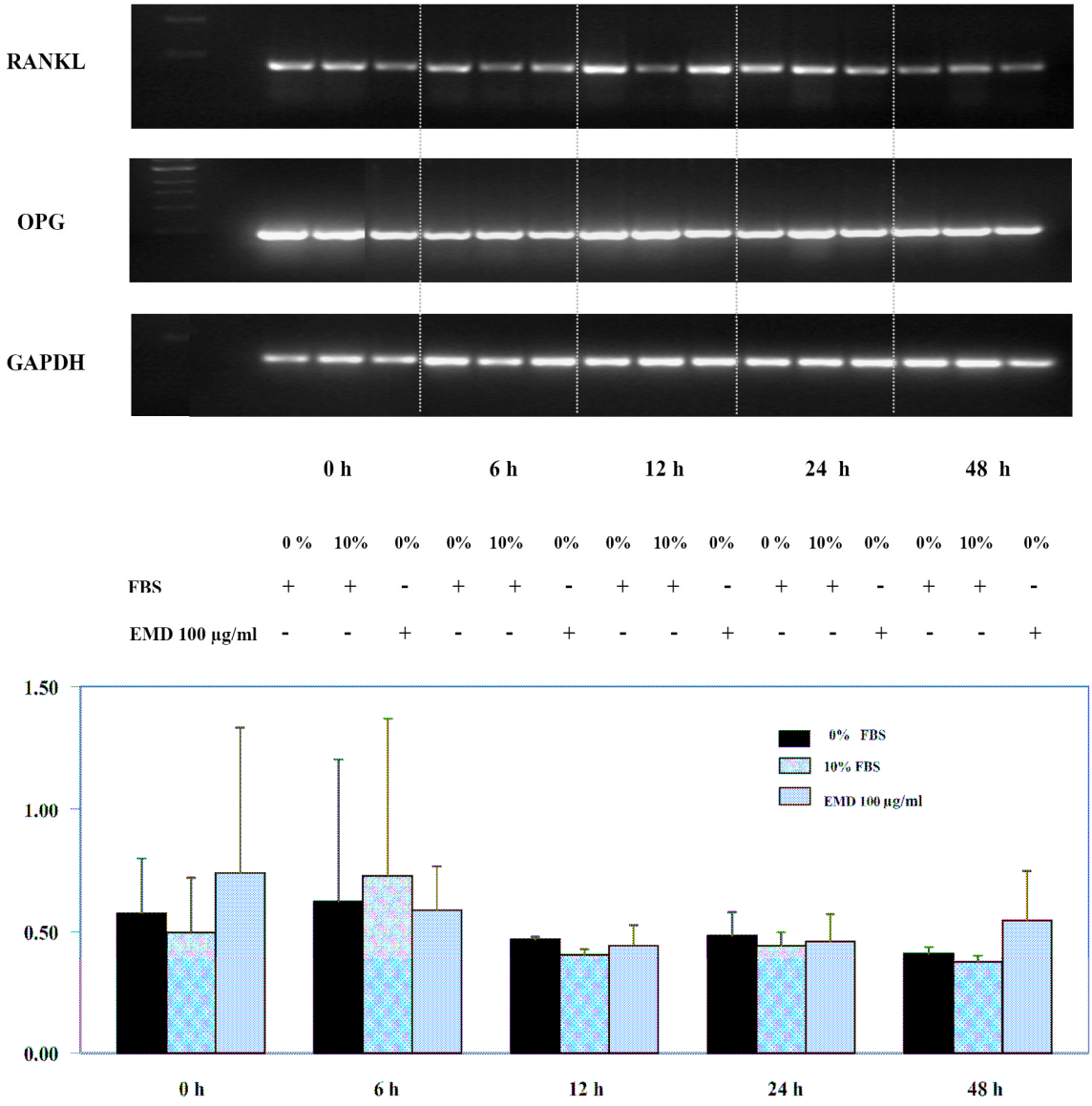
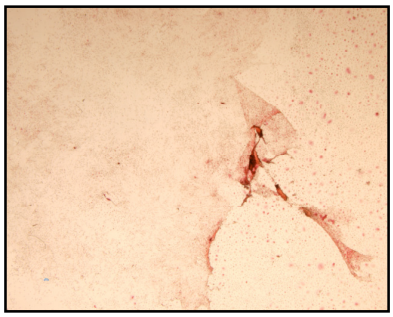


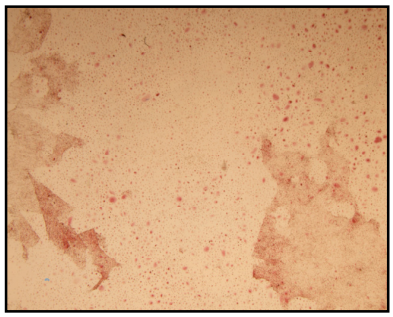
Figure 3.3 Graphical representation of RANKL/OPG ratio in osteoblast-like cell MG-63 treated with 0% FBS, 10% FBS and EMD 100 µg/ml for 0, 6, 12, 24 and 48 h, Each value represents mean ± SD of duplicate cultures.

3.2 Osteoclast formation

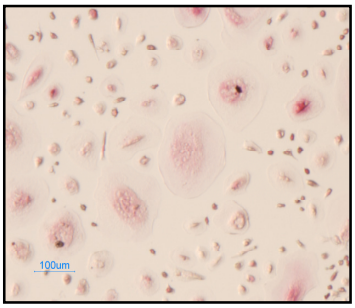
Osteoclast-like cell formation was analyzed in the cocultures of MG63 and PBMCs in the supplemented with or without EMD 100 μg/ml using TRAP staining. Osteoclast-like cells were identified as TRAP-positive cells with three or more nuclei. TRAP-positive cells were stained as dark red (Fig.3.4). When PBMCs were co-cultured with osteoblast-like cells MG63 in the presence of EMD, there was a significant increase in the number of osteoclast-like cells (p <0.05) (Fig.3.5).



A. Control group



B. EMD 100 μg/ml



C. Osteoclast-like cells

Figure 3.4 Characteristics of osteoclasts expressed by multinucleated cells formed in the cocultures of osteoblast-like cell MG-63 with either human peripheral blood mononuclear cells (PBMCs). Osteoblast-like cell MG-63, 6x106cells/well were cocultured with PBMCs, 4x106cells/well in 2 ml of DMEM containing 10% FBS in 24-well plates. 10-8M 1α, 25(OH)2D3 and 10-7M dexamethasone were added to all the cocultures with human PBMCs. After 14 days, cultures were fixed and stained for TRAP (A-C). Bar, 100 mm.

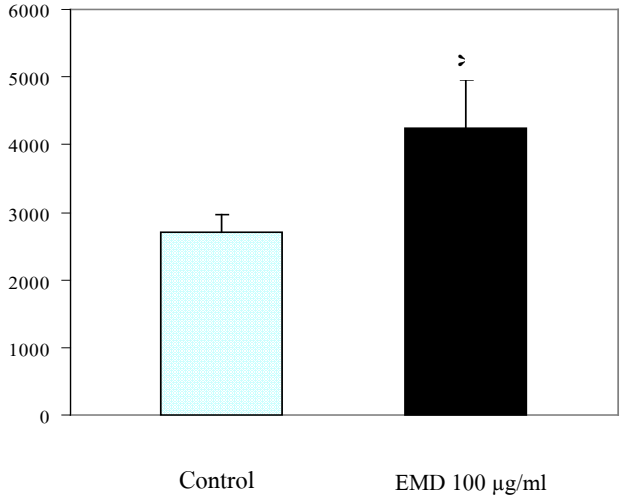


Figure 3.5 Effect of EMD on osteoclast formation in osteoblast-like cell MG-63 with either human peripheral blood mononuclear cells (PBMCs) for 2 weeks. EMD 100 µg/ml treatment significantly increased osteoclastogenesis activity when compared to control group. *P < .05.