**Extenuatory Effects of OP-1 on PMMA challenged MC3T3-E1 Cells In Vitro**

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**Introduction:** Osteolysis induced by polyethylene wear debris from orthopaedic implants mediates prosthetic loosening. Wear particles stimulate inflammatory cells such as macrophages and fibroblasts which inhibit osteoblastic differentiation of osteoprogenitors and mesenchymal stem cells, subsequent bone resorption in the peri-prosthetic tissue. Because mesenchymal stem cells and osteoprogenitors are the precursors to osteoblastic differentiation in the bone marrow, their viability is crucial to bone regeneration in the implant bed. Bone morphogenetic protein-7 (BMP-7, also known as osteogenic protein-1 or OP-1) is an osteogenic factor that stimulates maturation of mesenchymal osteoprogenitor cells to osteoblasts. This study tested the hypothesis that the addition of OP-1 (BMP-7) can mitigate the inhibitory effects of PMMA particles on MC3T3-E1 osteoprogenitor cells in vitro.

**Materials/Methods:** MC3T3-E1 osteoprogenitor cells (ATCC) in 12-well plates were induced to differentiate in osteogenic α-MEM containing ascorbic acid (50μg/ml) and β-glycerophosphate (10 m*M*). MC3T3-E1cells were challenged with PMMA particles (1-10 μm, Polysciences) at concentrations of 0.300, 0.150, and0.075% v/v on the first day of growth in osteogenic medium. Cells were treated with OP-1 (200 ng/ml) on the first or fourth day of growth in osteogenic medium according to the experimental setups summarized in Table 1. The quantity of mineralization in culture was measured after a 20-day culture period. Mineralized matrix was stained by incubating cultures in 5% silver nitrate solution under UV light for 1 hr. Stained areas were quantified using the software program NIH Image and expressed as a percentage of the total well area. ANOVA and Fishers post hoc analysis were performed. P-values < 0.05 were considered significant.

Table 1: Experimental conditions with PMMA challenged MC3T3-E1 cells

| Experiment | Osteogenic media | PMMA addition * | OP-1 addition * |
|---|---|---|---|
| 1 | Yes | Day 1-20 | None |
| 2 | Yes | Day 1-20 | Day 1-20 |
| 3 | Yes | Day 1-20 | Day 4-20 |
| 4 | Yes | Day 1-20 | Day 1-4 |
| 5 | Yes | Day 4-20 | None |
| 6 | Yes | Day 4-20 | Day 1-20 |

* Days 1 and 4 in Table 1 represent the first and fourth days of growth in osteogenic medium.

**Results:** PMMA particles suppressed the mineralization of the MC3T3-E1 cells in a dose dependent manner. Addition of OP-1 mitigated this suppressive effects at all the PMMA doses tested. Interestingly, the presence of OP-1 during the first 4 days of PMMA exposure yielded the same level of mineralization as addition of OP-1 for other prolonged durations (Figure 1). The same effect was also observed with cells at a more mature stage, when the cells were allowed to differentiate for 4 days before being exposed to PMMA particles and OP-1 (Figure 2)

**Discussion:** This study has shown that OP-1 can mitigate the inhibitory effects of PMMA particles on MC3T3-E1 osteoprogenitors. OP-1 appeared to influence mineralization of the cells studied at various stages of maturation, when exposed to PMMA particles. Local treatment with OP-1 may represent a new therapeutic candidate for the treatment of particle-associated bone loss.

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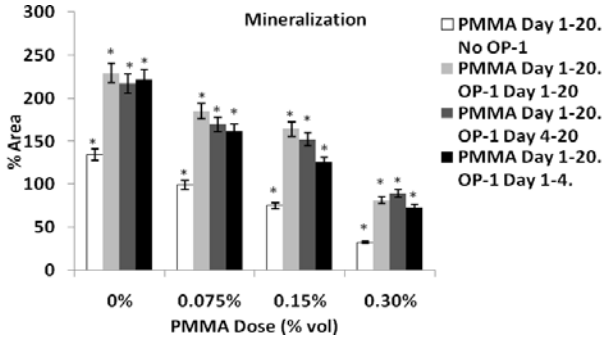


**Figure 1**: Dose dependent effects of OP-1 on MC3T3-E1 osteoprogenitors challenged with PMMA particles.

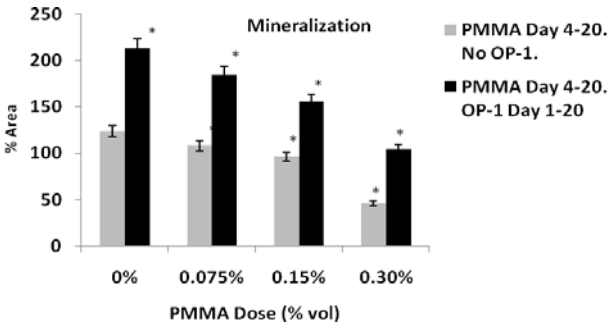


**Figure 2**: Dose dependent effects of OP-1 on MC3T3-E1 osteoprogenitors challenged with PMMA particles.