Polymethylmethacrylate Particles Inhibit Osteoblastic Differentiation of MC3T3-E1 Osteoprogenitor Cells
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Introduction: Polymethylmethacrylate (PMMA) particles have been previously shown to inhibit the differentiation of osteoprogenitors in murine marrow stromal cell cultures [1]. However, the heterogeneous marrow stromal cell population contains hematopoietic cells such as monocyte-macrophages, which may contribute to the inhibitory response of osteoprogenitor cells to particles. The purpose of this study was to determine whether the inhibitory effects of PMMA particles could be reproduced in pure osteoprogenitor populations. We examined the dose-dependent effects of PMMA particles on the differentiation of MC3T3-E1 cells, a murine osteoprogenitor cell-line, and determined whether these cells release soluble factors that can inhibit MC3T3-E1 osteogenesis after exposure to PMMA particles.

Materials and Methods: Confluent MC3T3-E1 subclone 14 osteoprogenitors (American Type Culture Collection) in 12-well plates were induced to differentiate in osteogenic α-MEM containing 50 μg/ml ascorbic acid and 10 mM β-glycerophosphate. These cells were treated with PMMA particles (1-10 μm, Polysciences) at concentrations of 0.038, 0.075, 0.150, 0.300, and 0.600% v/v on the first day of differentiation in osteogenic medium. In a separate experiment, MC3T3-E1 cells were grown in conditioned medium taken from confluent MC3T3-E1 cultures that had been challenged with 0.300% v/v PMMA particles for 24 hrs; control cells were grown in conditioned medium taken from confluent MC3T3-E1 cultures unexposed to particles. All cultures were assessed for mineralization, cell number, alkaline phosphatase activity, and osteocalcin production after 20 days of treatment with particles or conditioned medium. Mineralized matrix was stained with 5% silver nitrate solution under UV light for 1 hr. The total area of stained matrix was measured with the software program NIH Image and expressed as a percentage of the culture well area. Cell number was represented by the quantity of DNA, measured using the PicoGreen dsDNA Quantitation Kit (Molecular Probes). Alkaline phosphatase activity was measured using the QuantiChrome Alkaline Phosphatase Assay Kit (BioAssay Systems). Osteocalcin in medium samples was measured using the Mouse Osteocalcin ELISA EIA Kit (Biomedical Technologies). All data was statistically analyzed using ANOVA and Fisher’s PLSD, with p-values < 0.05 regarded significant.

Results: MC3T3-E1 cultures challenged with PMMA particles at doses of 0.038, 0.075, 0.150, 0.300, and 0.600% v/v respectively showed a 37.1, 79.8, 89.7, 99.9, and 99.9% reduction in mineralization; a 3.1, 9.7, 12.0, 99.9, and 99.9% reduction in cell number; and a 12.4, 22.8, 54.7, 99.9 and 99.9% reduction in alkaline phosphatase activity (Figure 1A). Osteocalcin production, however, was not significantly affected by particles at all doses tested (Figure 1B). Particle phagocytosis was evident as observed under light microscopy. Cells grown in conditioned medium from particle-treated MC3T3-E1 cultures showed a significant 54.7% decrease in mineralization compared to control cells grown in conditioned medium from MC3T3-E1 cultures unexposed to particles (Figure 2A). However, no significant changes in cell number, alkaline phosphatase activity, or osteocalcin production were observed (Figure 2AB).

Discussion: This study has shown that PMMA particles inhibit the differentiation of MC3T3-E1 osteoprogenitors in a dose-dependent fashion, with respect to mineralization, proliferation, and alkaline phosphatase activity. Reduction of cell number and alkaline phosphatase activity occurs gradually over the low particle dose range (0.038-0.150% v/v) but dramatically at high particle doses (≥ 0.300% v/v). Osteocalcin production, however, is unaffected by particle challenge. MC3T3-E1 cells also produce soluble factors that can inhibit mineralization but not differentiation or proliferation. However, the magnitude of inhibition achieved with soluble factors is much less than that resulting from direct particle contact/exposure. PMMA particles have been previously shown to inhibit the differentiation of osteoprogenitors in heterogeneous murine marrow stromal cell cultures in a similar dose-dependent fashion [1]. This study confirms that the inhibitory effects of PMMA particles observed with heterogeneous marrow stromal cell cultures are also observed with pure osteoprogenitor populations.


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