Ultrahigh Molecular Weight Polyethylene Wear Debris Inhibits Osteoblastic Differentiation of Bone Marrow Osteoprogenitors and MC3T3-E1 Preosteoblasts In Vitro

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Introduction: Osteolysis and prosthetic loosening may result from the biological effects of polyethylene wear debris. Polyethylene particles not only trigger inflammation and bone degradation, but also inhibit bone formation through the suppression of osteoblast function. However, whether polyethylene particles also inhibit osteoprogenitor differentiation is unknown. In this study, we examined the effects of ultrahigh molecular weight polyethylene (UHMWPE) particles on the ability of primary murine bone marrow osteoprogenitors and MC3T3-E1 cells, a murine osteoprogenitor cell-line, to differentiate into osteoblasts in vitro.

Materials and Methods: UHMWPE particles (0.5 μm ± 0.2 μm) generated from wear simulator tests performed at the Hospital for Special Surgery were isolated from serum by previously established methods [1]. UHMWPE particles were coated on the culture well surfaces of 12-well plates at concentrations of 0.038, 0.075, 0.150, 0.300, and 0.600% v/v with a layer of type I collagen. Control wells (0.00% v/v particles) were covered with a layer of type I collagen without UHMWPE particles. MC3T3-E1 subclone 14 preosteoblasts (American Type Culture Collection) and primary bone marrow cells isolated from the femur and tibia of C57BL/Ka mice were cultured on these particle-coated plates. MC3T3-E1 cells were grown in osteogenic α-MEM containing 50 μg/ml ascorbic acid and 10 mM β-glycerophosphate. Bone marrow cells were grown in osteogenic DMEM containing the same osteogenic factors with the addition of 0.1 μM dexamethasone. Cultures were assessed for mineralization, cell number, alkaline phosphatase activity, and osteocalcin production after 20 days. 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 EIA ELISA Kit (Biomedical Technologies). All data was statistically analyzed using ANOVA and Fisher's PLSD, with p-values < 0.05 considered significant.

Results: MC3T3-E1 preosteoblasts (Figure 1A-B-C-D) and primary bone marrow osteoprogenitors (Figure 2A-B-C-D) exposed to UHMWPE particles showed a dose-dependent decrease in mineralization, cell number, alkaline phosphatase activity, and osteocalcin production, with complete suppression of these outcome parameters observed at particle concentrations ≥ 0.150% v/v.

Discussion: This study has shown that UHMWPE particles inhibit the differentiation of primary bone marrow osteoprogenitors and MC3T3-E1 preosteoblasts in a dose-dependent fashion, with complete suppression of osteogenesis occurring at particle doses ≥ 0.150% v/v. Previous studies have shown that polymethylmethacrylate (PMMA) particles inhibit the differentiation of osteoprogenitors in heterogeneous bone marrow cell cultures, which contain hematopoietic cells and precursors that may influence the response of osteoprogenitor cells to particles [2]. This study confirms that the inhibitory effects of PMMA particles on the differentiation of osteoprogenitors in heterogeneous marrow stromal cell cultures are also observed with polyethylene wear debris. This study also confirms that these inhibitory effects can be reproduced in pure osteoprogenitor populations such as the MC3T3-E1 cell-line. The mechanism of polyethylene particle-induced implant loosening may therefore involve not only inflammation and bone degradation, but also decreased bone formation by osteoblasts due to the inhibition of osteoprogenitor differentiation.


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FIGURE 1. Figure 1: Dose-dependent effects of UHMWPE particles on MC3T3-E1 preosteoblasts; *p-value < 0.05 vs. control (0.000% v/v particles)

FIGURE 2. Figure 2: Dose-dependent effects of UHMWPE particles on bone marrow osteoprogenitors; *p < 0.05 vs. control (0.000% v/v particles)