Introduction: Orthopedic wear debris has been implicated as a potential inhibitory factor of stem cell osteogenesis. Given that the stability of an implant depends on adequate bone formation, reductions in osteogenesis by wear debris particles may result in accelerated osteolysis and implant loosening. In this study, we treated primary human mesenchymal stem cells (hMSCs) and the murine MC3T3-E1 osteoprogenitor cell line with polymethylmethacrylate (PMMA) particles and determined the effects of these materials on their ability to differentiate into osteoblasts in culture. We looked at the effects of particles on proliferation, transcription factor expression, osteoblast protein production, and matrix mineralization.

Materials/Methods: hMSCs and growth medium were purchased from Lonza (Walkersville, MD) and MC3T3-E1 osteoprogenitors from ATCC (Manassas, VA). hMSCs were derived from human bone marrow and positively selected for CDs 29, 44, 105, and 166, and negatively for CDs 14, 34, and 45. hMSCs and MC3T3-E1 cells were induced to undergo osteogenesis in vitro by addition of osteogenic factors ascorbic acid (50 µg/mL), β-glycerophosphate (10 mM), and dexamethasone (0.1 µM, for hMSCs) to medium. PMMA particles 1-10 µm in size from Polysciences (Warrington, PA) were concurrently added to cultures on this same day of osteogenic induction (day 0) at concentrations of 0.000, 0.075, 0.150, and 0.300% v/v. mRNA was extracted from cells during the first 10 days of culture and assessed by Q-PCR for expression of alkaline phosphatase (ALP) and collagen types 1A1 and 1A2 in hMSCs, and Runx2, osterix, Dlx5, and Msx2 in MC3T3-E1 cells. Primers and Q-PCR reagents were purchased from Applied Biosystems (Carlsbad, CA). hMSCs were also assessed for ALP production by enzyme activity assay of cell lysate with p-nitrophenylphosphate, and for proliferation by cell count, during the first 10 days of culture. Mineralization on the fourth week of culture was assessed by reaction of extracted matrix calcium with o-cresolphthalein. Post hoc tests and ANOVA were used to calculate statistical differences, with p-value < 0.05 considered significant.

Results: hMSCs treated with PMMA particles showed significant dose-dependent reductions in cell proliferation (Fig 1), ALP production (Fig 2), and mRNA expression of collagen types 1A1 and 1A2 (Figs 3 and 4) and ALP (Fig 5) throughout the first 8-10 days of culture. Mineralization as assessed by matrix calcium content on the fourth week of culture was significantly reduced at all particle doses tested (Fig 6). MC3T3-E1 cells also showed significant dose-dependent reductions in mRNA expression of transcription factors Runx2 (Fig 7), osterix (Fig 8), and Dlx5 (Fig 9); mRNA expression of Msx2 (Fig 10), an antagonist/repressor of Dlx5-mediated osteogenesis, remained relatively unaffected. Both hMSCs and MC3T3-E1 cells showed evidence of particle phagocytosis as observed by light microscopy (Figs 11 and 12).

Discussion: This study has demonstrated that PMMA particles inhibit the osteogenic differentiation of hMSCs and MC3T3-E1 osteoprogenitor cells. This inhibitory effect is indicated by the dose- and time-dependent decreases in cell proliferation, ALP and collagen expression, and matrix calcification in hMSCs, and in the expression of osteogenic transcription factors Runx2, osterix, and Dlx5 in MC3T3-E1 cells. Clinically, the inhibition of osteogenic differentiation of MSCs and osteoprogenitors by wear debris particles may result in diminished osteoblast production and bone formation, with resultant acceleration of osteolysis. Further studies will involve testing potential therapeutic agents to mitigate the adverse effects of PMMA particles on stem cell osteogenesis and to elucidate the molecular mechanism of this inhibitory effect.

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