Polymethylmethacrylate Particles Inhibit Runx2, Osterix, Dlx5, and β-catenin Expression in Osteoprogenitor Cells

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Statement of Purpose: Osteoprogenitor differentiation is regulated by the transcription factors Runx2, osterix, and Dlx5, and the transcriptional activator β-catenin. PMMA (polymethylmethacrylate) particles inhibit the osteogenic differentiation, maturation and mineralization of murine osteoprogenitors,[1] but the molecular mechanisms of this suppression are unknown. In this study, we determined whether the gene expression of the transcription factors Runx2, osterix, Dlx5, and β-catenin in murine MC3T3-E1 osteoprogenitor cells are inhibited by PMMA particles.

Methods: MC3T3-E1 subclone 14 osteoprogenitor cells (ATCC) were expanded to confluency in ascorbic acid-free α-MEM for 3 days in 12-well plates at an initial concentration of 1 x 10^5 cells/well. MC3T3-E1 cells were then cultured in osteogenic α-MEM containing ascorbic acid (50 μg/mL) and β-glycerophosphate (10 mM). Cells were simultaneously treated with PMMA particles (1-10 μm, Polysciences) at concentrations of 0.00, 0.15, 0.30, and 0.60% v/v on this first day of differentiation in osteogenic culture. RNA was extracted from cells by the TRIzol method each day throughout the first 6 days of culture, reverse transcribed into cDNA, and quantified by real-time PCR using TaqMan Mastermix reagents and primers for mouse Runx2, osterix, Dlx5, and β-catenin (Applied Biosystems). RNA levels were normalized to 18S expression. Statistical analysis was performed using post hoc tests and ANOVA with significance at $p < 0.05$.

Results: MC3T3-E1 cells treated with PMMA particles showed a significant dose-dependent decrease in Runx2, osterix, and Dlx5 expression at all days of measurement (Figures 1-3), and from days 1-4 for β-catenin (Figure 4).

Conclusions: This study has shown that PMMA particles inhibit gene expression of Runx2, osterix, Dlx5, and β-catenin in MC3T3-E1 osteoprogenitor cells. Inhibition of osteoprogenitor differentiation and maturation by PMMA particles may therefore be mediated by interference with transcription factors that regulate osteogenesis.

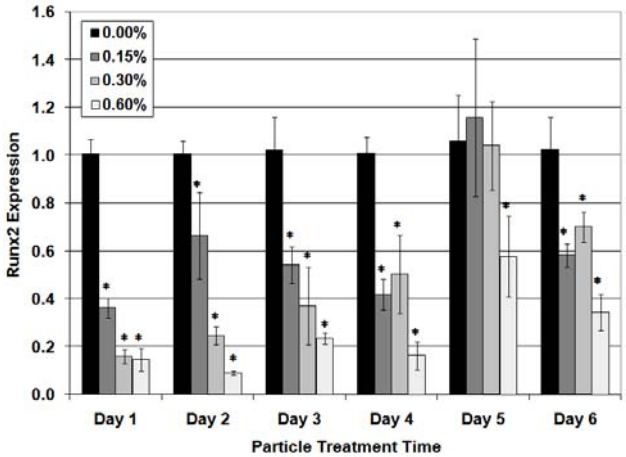

Figure 1. PMMA particle effects on Runx2 expression. N=4, *p < 0.05 vs. control (0.00% v/v particles).

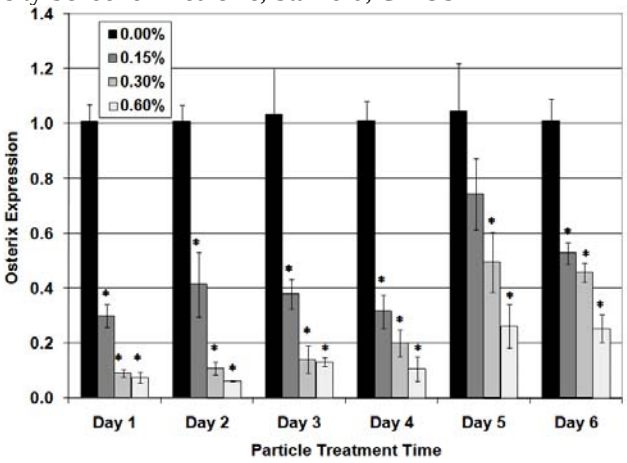

Figure 2. PMMA particle effects on osterix expression. N=4, *p < 0.05 vs. control (0.00% v/v particles).

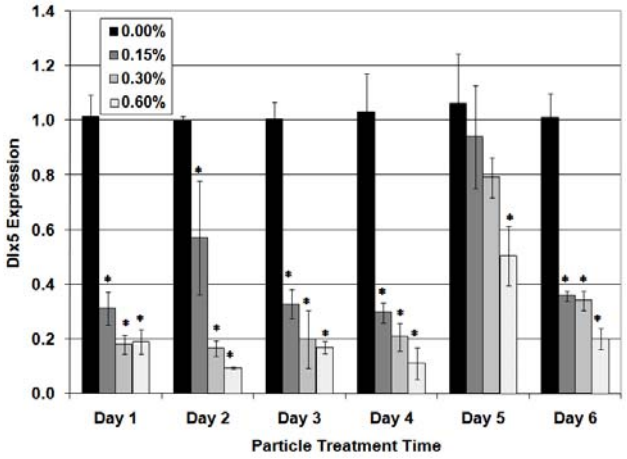

Figure 3. PMMA particle effects on Dlx5 expression. N=4, *p < 0.05 vs. control (0.00% v/v particles).

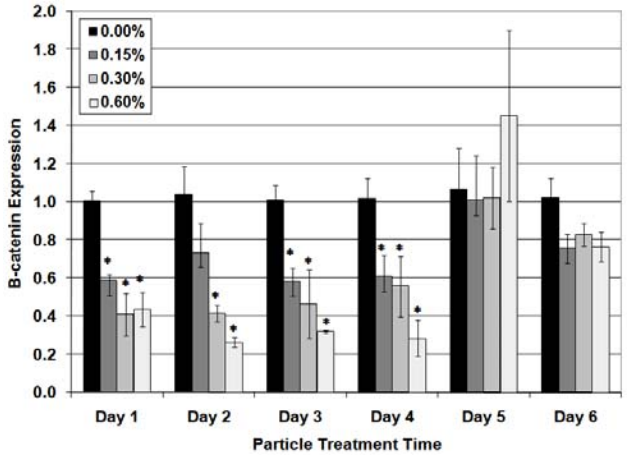

Figure 4. PMMA particle effects on β-catenin expression. N=4, *p < 0.05 vs. control (0.00% v/v particles).

References: 1. Chiu R. J Orthop Res. 2008; 26: 932-936.

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