Polymethylmethacrylate Particles Inhibit Human Mesenchymal Stem Cell Osteogenic Differentiation

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Statement of Purpose: Polymethylmethacrylate particles (PMMA) have been shown to inhibit the osteogenesis of murine primary osteoprogenitors and pre-osteoblast cell lines [1]. However, the effects of PMMA particles on human mesenchymal stem cell (hMSC) osteogenesis have not been investigated. In this study, we examined the effects of PMMA particles on the ability of hMSCs to differentiate into osteoblasts with respect to proliferation, alkaline phosphatase and type 1 collagen expression, and matrix mineralization.

Methods: Bone marrow-derived hMSCs were purchased from Lonza (Walkersville). These cells were positively selected for mesenchymal markers CDs 29, 44, 105, and 166, and negatively selected for hematopoietic markers CDs 14, 34, and 45. hMSCs were seeded in 12-well plates at an initial cell density of 5000 cells/cm^2 for 24 hrs in hMSC growth medium (Lonza), then induced to undergo osteogenic differentiation by addition of ascorbic acid (50 µg/ml), dexamethasone (0.1 µM), and β-glycerophosphate (10 mM) to medium. hMSCs were simultaneously treated with PMMA particles 1-10 µm in diameter (mean 6.0 µm, Polysciences) on this first day of osteogenic induction (day 0) at doses of 0.000, 0.075, 0.150, and 0.300% v/v. Cells were observed under the microscope for evidence of particle phagocytosis and counted under hemocytometer for proliferation. Alkaline phosphatase (ALP) production was measured by qRT-PCR for RNA expression, by enzyme reaction with p-nitrophenylphosphate for protein activity, and by staining with BCIP/NBT ALP Staining Kit (BioAssay Systems) for cell surface expression. Type 1 collagen expression was measured by qRT-PCR, using reagents and human primers from Applied Biosystems. Proliferation, ALP production, and collagen expression were measured at 2-day intervals during the first 8-10 days of culture. Mineralization on the fourth week of culture was assessed by measuring matrix calcium content using a calcium colorimetric assay kit (BioVision) after calcium extraction in 0.60 N HCl. Statistical analysis was performed by ANOVA and post hoc tests.

Results: hMSCs treated with PMMA particles showed a significant dose-dependent decrease in proliferation (Fig 1), ALP activity (Fig 2), ALP RNA expression (Fig 3), and collagen type 1A1 and 1A2 RNA expression (Figs 5 and 6) throughout days 1-10 of culture. Mineralization as measured by matrix calcium content on the fourth week of culture was also significantly reduced (Fig 4). hMSCs showed evidence of particle phagocytosis within 24 hrs of treatment (Fig 7) and significant decreases in cell surface expression of ALP (Fig 8).

Conclusions: This study has shown that PMMA particles inhibit hMSC differentiation into osteoblasts, as indicated by the dose-dependent reductions in proliferation, ALP and collagen expression, and mineralization. Clinically, osteolysis and implant loosening of total joint arthroplasty results not only from the effects of implant wear debris on inflammatory cells, but also from reduced bone formation from inhibited osteogenesis of mesenchymal stem cells. Future studies will test potential therapeutic agents to mitigate the inhibitory effects of implant wear debris on progenitors and stem cells in the early osteogenic lineage.

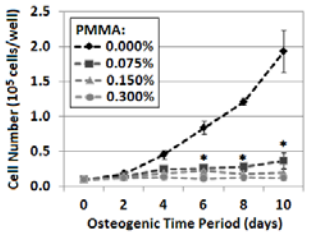

Figure 1.

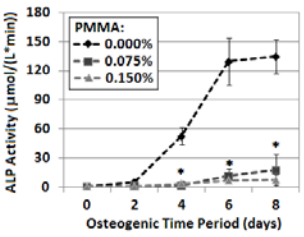

Figure 2.

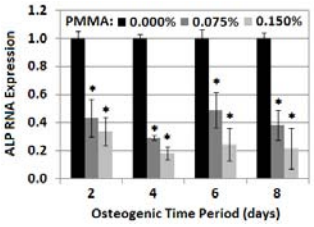

Figure 3.

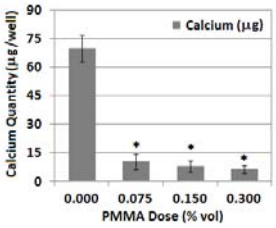

Figure 4.

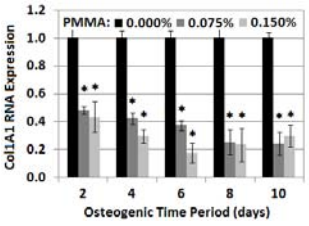

Figure 5.

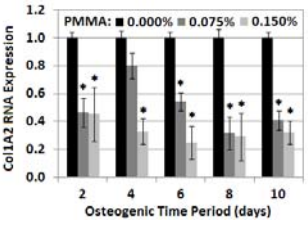

Figure 6.

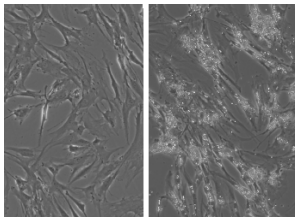

Figure 7.

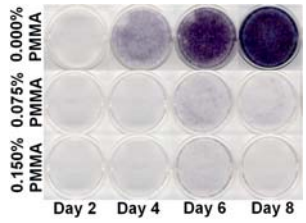

Figure 8.

Figs 1-6: N=4, * $p < 0.05$ vs. control (0.00% particles) in the same day of measurement. **Fig 7:** Light microscopy images of hMSCs with no particles (left) and hMSCs with phagocytosed particles (right).

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References: 1. Chiu R. J Orthop Res. 2008; 26: 932-6.