Osteoprogenitors Are Inhibited by Direct Exposure to Polymethylmethacrylate Particles or by Soluble Factors Released from Particle-Activated Macrophages

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Statement of Purpose: The inhibition of osteogenesis by orthopedic wear debris may be due to a direct effect of particles on osteoblast progenitors or an indirect effect of inhibitory factors released from particle-activated cells. This study determined whether polymethylmethacrylate (PMMA) particles and conditioned medium from particle-activated macrophages, osteoblasts, and marrow stromal cells inhibit osteoblast differentiation and mineralization.

Methods: Murine marrow stromal cells (MSCs) and MC3T3-E1 subclone 14 preosteoblasts were cultured in osteogenic medium containing 50 μg/ml ascorbic acid and 10 mM β-glycerophosphate (medium for MSCs also contained 0.1 μM dexamethasone). MC3T3-E1 cells in their undifferentiated state were allowed to proliferate to confluency for 3 days prior to growth in osteogenic medium. MSCs and MC3T3-E1 cells were treated with PMMA particles at a dose of 0.30% v/v on the first day of growth in osteogenic medium. Separate cultures of MSCs were incubated in conditioned medium collected from the following sources: (1) Raw264.7 macrophage cultures challenged with PMMA particles (0.30% v/v) for 24 hrs at 95-100% confluency; (2) cultures of fully differentiated mineralizing osteoblasts that were derived from MSCs grown in osteogenic medium for 10 days, and treated with PMMA particles (0.30% v/v) for 72 hrs at 70%-90% confluency; and (3) cultures of early differentiating MSCs treated with PMMA particles during their first 5 days of growth in osteogenic medium. Fresh MSCs were incubated in the conditioned medium with the addition of an equal volume of fresh osteogenic medium to ensure an adequate supply of nutrients. Negative control MSCs were grown under the same experimental conditions, but received conditioned medium from cultures that were not exposed to particles. MSCs were treated with particles or conditioned medium for 15 days; MC3T3-E1 cells were treated with particles for 30 days. Cultures were stained for mineralized nodules/matrix by the von Kossa method and for alkaline phosphatase-positive colonies using BCIP/NBT alkaline phosphaase staining kit (Vector). The area of stained matrix/nodules and colonies was measured with NIH Image and expressed as a percentage of the well area. Statistical analysis of the data was performed using ANOVA and Fisher's PLSD.

Results/Discussion: MSCs and MC3T3-E1 cells directly exposed to PMMA particles showed a ≥ 95% reduction in mineralization compared to control cells not exposed to particles (Figure 1). Particle-treated MSCs also showed a ≥ 95% decrease in the quantity of alkaline phosphatase-positive colonies (Figure 2). MSCs grown in conditioned medium from particle-challenged Raw264.7 macrophage cultures showed a significant 84% reduction in mineralization (Figure 1) but a non-significant change in the quantity of alkaline phosphatase-positive colonies (Figure 2). MSCs grown in conditioned media from particle-challenged cultures of mature osteoblasts and early differentiating MSCs showed similar levels of mineralized nodules and alkaline phosphatase-positive colonies as control cells grown in conditioned medium from cultures not exposed to particles (Figures 1, 2).

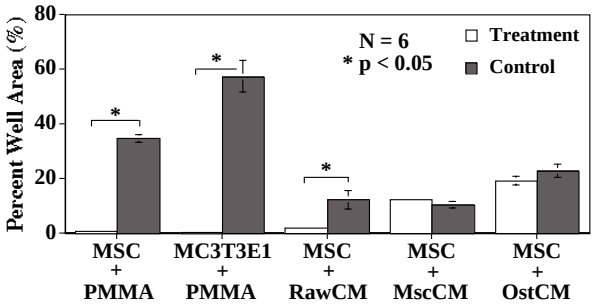

Figure 1. Mineralized Nodules/Matrix

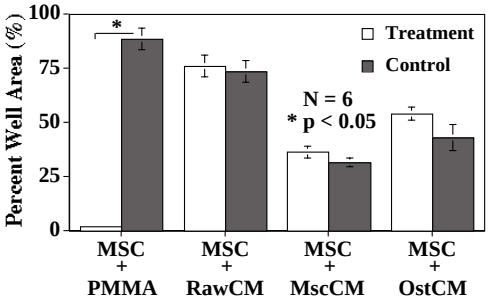

Figure 2. Alkaline Phosphatase-Positive Colonies

Figures: Labels on the horizontal axis represent the cell type (MSC or MC3T3-E1) plus the treatment received. Particle treatment represented by "PMMA;" conditioned medium treatment represented by "RawCM" (Raw264.7 macrophages), MscCM (early differentiating MSCs), and OstCM (mature osteoblasts). Comparisons are between experimental groups (white bars) and control groups (dark bars) grown without particles or incubated in conditioned medium from cells not exposed to particles.

Conclusions: This study demonstrated that exposure of MSCs or MC3T3-E1 preosteoblasts to PMMA particles or to conditioned medium from particle-activated macrophages causes a significant inhibition of osteoblast differentiation and mineralization. Macrophages exposed to particles release proinflammatory factors that impair osteoblast development. In contrast, conditioned medium from particle-treated MSC-derived osteogenic cells in both the early and late stages of differentiation did not affect osteogenesis, which suggests that these cells did not release detrimental soluble inhibitory factors. Therefore, the suppression of osteoprogenitor differentiation appears to be a combined effect of direct exposure to PMMA particles and inhibitory factors released from particle-activated macrophages.

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