The Effects of PMMA on MG-63 Osteoblast Cell Function and Morphology

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Introduction: Much of the focus on the long-term biocompatibility of implant materials has centered on the macrophage and it's response toward wear debris particles. There is increasing evidence that wear debris particles that are present in periprosthetic tissues have direct effects on osteoblasts. Particles resulting from polymethylmethacrylate (PMMA) cements used for fixation may also be involved directly in aseptic loosening of implants. However, it is not known if these particles have a direct or indirect effect on bone formation. The objective of this study was to determine the effect of PMMA particle number on osteoblast cells.

Material and Methods:

Cell Culture- MG-63 bone cells were cultured and treated with low (0.01 mg/mL), medium (0.1 mg/mL), and high (0.5 mg/mL) doses of PMMA solution for 24, 48, and 72 hour time periods.

Cell Count- The cells were collected into labeled microcentrifuge tubes. The tubes were centrifuged for 10 min. @ 1000 rpm, and the cells resupened in then 300 µl phosphate buffered saline. Cell number was determined by hemacytometer counting method.

Staining- Coverslips were stained with hematoxylin and eosin (H&E) using standard laboratory procedures. The slides were then analyzed using light microscopy and digitized.

Protein Assay: Cellular protein levels were determined using Pierce BCA reagent

MDA Assay: An MDA standard was prepared by serial 2-fold dilution to obtain concentrations ranging from 50µM-0µM MDA. Either 100µL of standard solution or cell suspension were added to a labeled glass tube. Five hundred microliters of TCA were added to all the tubes, and after a short (1-minute) incubation, 500 µL of TBA were added to all tubes. These tubes were placed in a water bath (100°C) for 45 minutes, cooled to room temperature, and then centrifuged at 2,500 rpms for 10 minutes. Following centrifugation, 100µL were withdrawn from each standard and sample and placed into wells on a 96 well assay plate. The plate was analyzed using the Spectra plate reader at a wavelength of 532 nm[4].

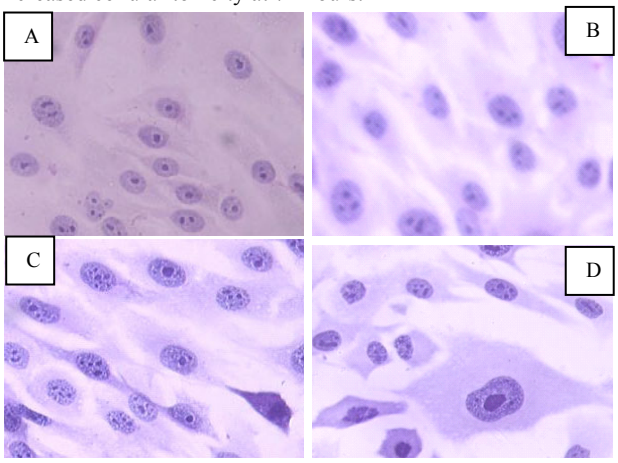
Statistics and Graphics: Descriptive statistics and analysis of variance were performed using Sigma Stat Software.

Results and Discussion:

Cell Count- Cells exposed to low and medium doses of PMMA at 24 hours had cell numbers similar to the control. The high dosage exposure resulted in a significant decrease in cell number as early as 24 hours ($p<0.05$). After 48 hours of exposure, the low dose was still similar to control untreated cells whereas the medium and high dose exposures showed decreases in cell number. After 72 hours of exposure, cells were still viable in the experimental groups; however, low and high dose exposuress showed a 50% reduction in cell number compared to control

MDA Assay- Following the 24 hour dosing period, significant cellular damage was seen for the cells expose to the high dose compared to the control. After 48 hours in culture, the medium and high dose exposed cells showed slight increases in cell damage compared to the control. By 72 hours in culture, increases in damage for all experimental groups were observed compared with the control. Overall, the high-dose PMMA exposure appeared to be most toxic to cells.

Morphology-: Morphological observations showed significant vacuole formation in all experimental groups. Also, there was an overall increase in cell size that coincided with increasing levels of PMMA with noted micronucleoli at the highest dosage. The increase in micronucleoli may be reflective of increased cellular toxicity at 72 hours.



Representative morphology of osteoblast like cells exposed to increasing concentrations of PMMA for 72 hours. A= Control, B=Low, C=Medium, and D=High

The effects of particle exposure on osteoblasts are well documented. Vermes and coworkers exposed osteoblasts with metal particles or polymeric particles, and showed reduced cell proliferation in a dose-dependent manner without affecting cell viability during a 24 hour period. Using fluorescent labeled particles, they were able to conclude that the MG-63 osteoblasts phagocytosed particulate wear debris. The results obtained in our study also reflect decrease cell numbers; however, we also demonstrated an increase in cell damage with PMMA exposure. Our results also show the ability of the cell to engulf PMMA particles in a dose dependent manner. Interestingly, our results indicate an increase in the number of micronuclei which is highly suggestive of increased genotoxicity of the cells. This genotoxicity by PMMA was also seen by Jensen *et al*. (1991) and Bigatti *et al.* (1994). Baggati and coworkers using human lymphocytes found PMMA particulate wear debris induced micronuclei in binucleated cells. Their study could not determine if PMMA bone cement or its polymerizing ingredients induced the increased frequency of micronucleus formation. Our study suggests that the PMMA particles themselves are effective in inducing micronuclei formation.

The results of our study as well as others have clearly showed that the bone cells can respond to material debris by increasing their ability to phagocytose the material as well as surmount an inflammatory response by increasing their production of cytokines. It is evident that the osteoblast cells play a more important role in osteolysis than once thought.

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