**In vitro** Macrophage response to cross-linked and conventional polyethylene particles

Smith, RA; Allen, B; Hauser, S

University of Tennessee; Department of Orthopaedic Surgery-Campbell Clinic, Christian Brothers University

**Introduction**

The new highly cross-linked ultra-high molecular weight polyethylene (xPE) has been developed in an effort to reduce wear, and therefore decrease the risk of osteolysis. Compared with conventional UHMWPE (cPE) the wear rates are lower and the particles that are produced are much smaller in size. While number and size of PE particles have been reported to play important roles in the degree of macrophage activation, there is no general consensus as to which size to dose ratio of particles within the phagocytosable range (<5um diameter), are maximally inflammatory. It is likely there are inherent differences of particles involving size, shape, material type, surface charge, surface texture, and hydrophobic/hydrophilic characteristics. However, further investigation of these properties is essential to eventually develop a scientific consensus.

PE may be inflammatory to periprosthetic tissue and bone in part because of its affinity for immunostimulatory byproducts termed pathogen-associated molecular patterns (PAMPs) from bacteria or endogenous inflammatory molecules such as complement and coagulation proteins that may play a role in osteolysis. Studies have demonstrated that endogenously or exogenously adsorbed PAMPs present on orthopedic wear particles stimulate macrophages to release cytokines known to increase osteoclastic activity. The goal of this study is to compare the cytokine response of macrophages to xPE and cPE particles *in vitro* in presence or absence of a common PAMP, lipopolysaccharide (LPS). The null hypothesis is that the two PE particle forms will cause the same cytokine response at different particle doses. This is to test the current popular idea that the xPE particles will cause less of a macrophage osteolytic response than the cPE particles.

**Materials and Methods**

IC21 mouse peritoneal macrophages and THP1 human monocytes were purchased from American Type Culture Collection. cPE and xPE were provided by Dr. Nadim Hallab, BioEngineering Solutions Inc. Particles were provided in ethanol with no detectable endotoxin (Pyrogen 5000 assay, BioWhittaker). Mean diameter for these particles were determined by Laser diffraction analysis and was 1.62 um for cPE and 0.68 um for the xPE. The 'LPS' particles were incubated in 0.78 µg/ml LPS (ultrapure LPS from E.coli, InvivoGen) at room temperature for 24 hours, and then washed with endotoxin-free water 3 times to remove the unbound LPS using 0.02 µm VectaSpin™ Micro centrifuge filters (Whatman International Ltd.). The hydrophobic LPS associated particles were dispersed in culture medium with 2% fetal bovine serum (FBS, Gibco). The macrophage cell number was approximately 200,000 per culture well, so for a 1 particle per cell (P/C) concentration there were about 200,000 per well. Cytokines including tumor necrosis factor alpha (TNFα) and interferlin one beta (IL-1β) were measured in the medium after 24 hour culture. T-tests were used to compare means between clean and LPS associated particles at same P/C doses and between cPE and xPE at same doses. A p<0.05 was considered significant.

**Results**

In this study neither cPE nor xPE particles decreased cell viability even at 250 particles per cell (data not shown).

**Discussion**

This study used clinically relevant sized cPE and xPE particles after cleaning of LPS and with LPS added, to assess cellular response. Our findings indicate no decreased cell viability with an accumulation of clean cPE or xPE particles, therefore these may be well tolerated *in vivo*. In this study TNFα and IL-1β levels were significantly increased when macrophages were exposed to LPS associated particles with increasing amounts correlated with increasing numbers of LPS bound particles. Particles without LPS elicited no cytokine secretion over baseline levels. These data also indicate that LPS associated cPE particles elicited a similar response for TNFα and IL-1β as LPS associated xPE, especially at high particle to cell numbers (100:1, 250:1) for both cell lines. Therefore we accept our null hypothesis that the two PE particle forms caused the same cytokine response at similar doses. The human THP-1 cell line used can vary in its response to activators such as LPS or PMA. Addition of PMA for 48 hours prior to addition of particles did not alter cytokine results in this study.

**Conclusion**

While the effects of a wear particle’s chemical properties on osteolytic response remain controversial, the prevailing inflammatory factor in this study was a PAMP (LPS). In these short-term *in vitro* experiments the number of polymer particles did not exhibit a significant difference in the macrophage cytokine response if the particles were clean. These results indicate that PE may be inflammatory to periprosthetic tissue and bone in part because of its affinity for PAMPs. The number and size of the particles (whether xPE or cPE) within this study had no effect on the cells inflammatory response if the particles were clean. The cytokine responses to both xPE and cPE LPS exposed particles were very similar indicating that the size of the particle (xPE being much smaller) did not influence cytokine response to the bound LPS.