Effect of UHMWPE Wear Debris Size, Dose, and Endotoxin on Macrophage Response

Carrie F. Alley\(^1\), Richard A. Smith\(^2\)

\(^1\) Smith & Nephew, Inc., Memphis, TN; \(^2\) University of Tennessee-Campbell Clinic Department of Orthopaedic Surgery, Memphis, TN

**Introduction:** Polyethylene particle size, number, and endotoxin have all been shown to contribute to the osteolytic response; however, research in this area is conflicting. Researchers have concluded polyethylene particles with a mean size from 0.24 μm to 7.2 μm are the most inflammatory size particle, depending on the number of particles per cell, and inflammatory response increases with particle number [1,2]. Other studies contradict these results by reporting that polyethylene particles without endotoxin generate little to no inflammatory response, while particles contaminated with endotoxin are very inflammatory [3,4]. The goal of this study is to determine which of these factors significantly contributes to inflammation by studying the release of inflammatory mediators by macrophages in vitro in response to UHMWPE wear debris generated from a hip simulator.

**Materials and Methods:** Conventional UHMWPE particles were isolated from hip simulator serum using established techniques [5]. A fraction of particles was then passed serially through filters with the following pore sizes: 12.0, 1.0, 0.4, and 0.05 μm for size separation. Particles were cleaned with four 10-ml washes of 10% Triton-X 100 before a sample of each particle fraction was bound with LPS (from E. coli, Sigma) [4]. Particle equivalent circular diameter (ECD) and number were characterized through SEM (JEOL JSM-6460LV, Tokyo, Japan) analysis. Each particle fraction was resuspended by sonicating the filter in 10 ml of RPMI 1640 medium (Sigma, St. Louis, MO) to create particle samples containing the number of particles which are representative of that generated by the hip simulator in each size range. While fewer particles are present in the large particle size samples as compared to the small particle size samples, the ratio of the two is the same as would be generated clinically. The “Clean” control sample was a filter without particles sonicated in endotoxin in free medium and the “LPS” control was an LPS-washed filter sonicated in medium. Endotoxin levels were measured using the Limulus Amebocyte Lysate (LAL) assay (Cambrex, East Rutherford, NJ).

IC-21 murine macrophages (ATCC TIB-186, American Type Culture Collection, Manassas, VA) were cultured in RPMI 1640 supplemented with 1% L-glutamine (Sigma), 0.13% gentamicin (Sigma), and 10% fetal calf serum (Sigma). Cells were seeded in 24 well plates at 2.5 x 10^5 cells per well and cultured for 24 hours. Serum was added to the particle fractions to allow them to contact the macrophages [6]. Each particle size was tested at three doses. The number of particles per cell for the high dose (H) of each particle fraction is given in Table 1. Dose H was diluted 1:5 and diluted 1:500 for Dose L. The TNF-α and IL-6 results for the “LPS” particle fractions were as all very high (>990 EU/ml). Endotoxin levels for Dose M of the “Clean” and “LPS” particle fractions are equivalent to the Dose H diluted 1:5 and diluted 1:500 for Dose L. The TNF-α and IL-6 results for the “Clean” particle fractions all showed low basal responses, similar to the control. The TNF-α and IL-6 results for the “LPS” particle fractions are given in Figures 1 and 2. There was a significant effect of both endotoxin level (p<0.0001) and test replicate (p<0.0001) for TNF-α and IL-6. There was no significant effect of particle dose or size tested in this study for either of the assays. Though test replicate was significant, the results did not appear to be physiologically relevant.

**Discussion:** While the effects of size and dose of polyethylene particles on osteolytic response remains controversial, the prevailing inflammatory factor in this study was endotoxin. In these in vitro experiments the size and number of UHMWPE particles made no significant difference in the macrophage cytokine response. These results indicate that polyethylene is inflammatory to periprosthetic tissue and bone in part because of its affinity for endotoxin.


---

**Table 1. Particles per Cell, Dose H**

<table>
<thead>
<tr>
<th>Particle Fraction</th>
<th>“0”</th>
<th>“0.05”</th>
<th>“0.4”</th>
<th>“1.0”</th>
<th>“12.0”</th>
<th>“Clean”</th>
</tr>
</thead>
<tbody>
<tr>
<td>Particles per Cell</td>
<td>300</td>
<td>730</td>
<td>447</td>
<td>162</td>
<td>102</td>
<td>no particle</td>
</tr>
</tbody>
</table>

---

**A general linearized nested model was used to determine the significance of the effect of the test variables (test replicate, particle size range, dose of particles, and endotoxin). Dose was nested within particle size and endotoxin was nested within dose.**

**Results:** The average ECD for the “All” particle fraction was 0.10 μm, 0.11 μm for “0.05”, 0.47 μm for “0.4”, 1.19 μm for “1.0”, and 8.44 μm for “12.0”. Endotoxin levels for Dose H of “Clean” particle fractions were all low (<0.27 EU/ml) and endotoxin levels for Dose H of “LPS” particle fractions were as all very high (>990 EU/ml). Endotoxin levels for Dose M of the “Clean” and “LPS” particle fractions are equivalent to the Dose H diluted 1:5 and diluted 1:500 for Dose L. The TNF-α and IL-6 results for the “Clean” particle fractions all showed low basal responses, similar to the control. The TNF-α and IL-6 results for the “LPS” particle fractions are given in Figures 1 and 2. There was a significant effect of both endotoxin level (p<0.0001) and test replicate (p<0.0001) for TNF-α and IL-6. There was no significant effect of particle dose or size tested in this study for either of the assays. Though test replicate was significant, the results did not appear to be physiologically relevant.

**Discussion:** While the effects of size and dose of polyethylene particles on osteolytic response remains controversial, the prevailing inflammatory factor in this study was endotoxin. In these in vitro experiments the size and number of UHMWPE particles made no significant difference in the macrophage cytokine response. These results indicate that polyethylene is inflammatory to periprosthetic tissue and bone in part because of its affinity for endotoxin.


---

**Figure 1. TNF-α (pg/ml) response for each “LPS” particle fraction at Doses H, M and L.**

**Figure 2. IL-6 (pg/ml) response for each “LPS” particle fraction at Doses H, M and L.**