CELLULAR RESPONSES TO ORTHOPAEDIC WEAR DEBRIS ARE INFLUENCED BY PARTICLE MORPHOLOGY

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Introduction
Total joint arthroplasty (TJA) is commonly used to alleviate the debilitating effects of arthritis. Nevertheless, up to 25% of TJA cases will require surgical revision due to implant failure. The generation of wear debris through articulation of the artificial joint is considered one of the prime factors promoting the chronic inflammation that leads to implant failure. Previous studies using uniformly shaped debris have identified that the particulate size, debris concentration, and material composition of the wear debris are factors that influence the degree of the inflammatory reaction that subsequently leads to the aseptic loosening of the prosthesis.

However, scanning electron microscopy (SEM) of debris isolated from periprosthetic tissue shows that wear particulates have neither a consistent shape nor texture. We have previously demonstrated that any deviation from a smooth round wear debris morphology results in a pronounced cellular activation and inflammatory cytokine production in a murine model of inflammation. To extend these findings to arthroplasty patients, we have developed a proliferation assay to examine the human cellular response to variations in wear debris morphology.

Materials and Methods
Polyethylene wear debris was recovered from periprosthetic tissue samples. Tissues were digested in chloroform/methanol, and then 5N NaOH at 65°C. Particles released during this process were separated into 3 fractions by sucrose gradient. Particles were washed in 70% ethanol and suspended to a final concentration of 2 X 10⁷ particles/ml in RPMI media supplemented with 5% FCS. All particle samples tested negative for endotoxin.

Shape and texture characteristics were evaluated using a S-2400 Hitachi scanning electron microscope and image analysis software. Particles were scored according to aspect ratio (AR) and texture features.

Thirty blood samples were acquired for the proliferation assay. In a 96 well plate patient cells and wear debris particles were combined at the concentrations of 1:1000, 1:100, and 1:10 for each group. Also added to the plate, synthetic (simulator-generated) UHMWPE (at the same concentrations as the tissue isolated polyethylene) and Concanavalin A (Con A), as a positive control for cell proliferation. After 6 days of particle incubation supernatant was recovered, and MTT/RPMI was added to the plate. After 6 hours, MTT/RPMI was removed and 10% SDS was added. Plates were incubated 24 hours, then assayed with a microplate reader at 590nm, and analyzed using softmax software.

Results
SEM images of debris were analyzed for aspect ratio, size, and texture. Scatter plot analysis of wear debris shape and texture revealed 4 unique populations characterized as: (1) smooth-round, (2) rough-round, (3) smooth-fibular, and (4) rough-fibular. Debris within an AR range of 1 to 2 was described as round. Fibular shaped debris had an AR range of 2 to 5. Similarly, debris with a smooth texture ranged from a score of 1 to 2.3. Debris with a score above 2.3 had a rough texture (Table 1).

Discussion
Our results suggest that texture and shape characteristics of wear debris strongly affect the cellular proliferate response. Any deviation from a smooth texture and round shape morphology in the stimulating particle resulted in a pronounced increase in cellular proliferation. The strongest response was associated with fibular shaped debris with a rough texture.

In comparison to synthetic UHMWPE particulates, wear debris that was fibular in shape or had a rough texture activated a more aggressive cellular response. Similar proliferate responses between Group 1, smooth-round debris, and synthetic UHMWPE suggest that morphological characteristics have a stronger influence than debris size on degree of cellular proliferation. This research shows the necessity to evaluate material biocompatibility with clinically relevant products. We propose to examine variances in cellular signals as a result of cellular exposure to wear debris with irregular morphology to extend these observations.

<table>
<thead>
<tr>
<th>Group</th>
<th>AR</th>
<th>Texture</th>
<th>Area (μm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>UHMWPE</td>
<td>1.25±0.04</td>
<td>1.34±0.03</td>
<td>3.62±0.50</td>
</tr>
<tr>
<td>1</td>
<td>1.73±0.06</td>
<td>1.92±0.07</td>
<td>1.17±0.03</td>
</tr>
<tr>
<td>2</td>
<td>1.71±0.06</td>
<td>2.94±0.11</td>
<td>1.15±0.03</td>
</tr>
<tr>
<td>3</td>
<td>3.03±0.25</td>
<td>1.98±0.10</td>
<td>1.13±0.02</td>
</tr>
<tr>
<td>4</td>
<td>3.59±0.50</td>
<td>2.74±0.28</td>
<td>1.22±0.15</td>
</tr>
</tbody>
</table>

Debris SEM analysis of synthetic UHMWPE debris revealed an AR and texture score similar to Group 1 wear debris; namely round and smooth. The synthetic UHMWPE debris size range was higher than wear debris from Groups 1, 2, 3, and 4. No difference in size range between Groups 1, 2, 3, and 4 was detected. Figure 1 shows the influence of wear debris morphology on proliferation. Debris from Group 4 provoked significantly higher responses than UHMWPE (p<0.001), Group 1 (p<0.001), Group 2 (p<0.004), and Group 3 (p<0.001). Groups 2 and 3 debris activated a more pronounced proliferate response compared synthetic UHMWPE (p<0.001 and p<0.009, respectively). No difference in proliferate response was observed between Group 1 and synthetic UHMWPE.

Figure 1: Influence of Wear Debris Morphology on Degree of Cellular Proliferation.