Evaluation of methods for periprosthetic tissue digestion and polyethylene wear particles analysis

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Introduction: Particles of polyethylene (PE) wear debris have been associated with loosening of hip implants and with osteolysis, which leads to implant failure. A number of studies have evaluated the physical properties and number of debris particles isolated from tissues adjacent to failed implants. As a result, there exist many proposed methods of tissue digestion for isolating polyethylene (PE) wear particles from retrieved periprosthetic tissue [1-3]. However, current studies and standards (e.g., ASTM F561-05a) provide limited guidance or comparative assessment of particle isolation techniques. The purpose of this study was to evaluate the efficiency of a variety of digestion methods.

Materials and Methods: Porcine tissue was obtained from six distinct regions of cadaveric porcine hip. After extraction of lipids with chloroform/methanol, the tissues were digested using either strong bases or enzymes to compare their efficiency. Acid digestion was ruled out because of acid-induced changes in particle morphology [4]. Solutions of sodium hydroxide, potassium hydroxide (Fisher Scientific, Hampton, NH), Proteinase K (Bioline, Randolph, MA) and Liberase Blendzyme 3 (Roche Applied Science, (Indianapolis, IN) were prepared. Each digestion method was assigned three different concentrations. Porcine hip samples (n=3) were randomly assigned to each test condition, as summarized in Table 1. One-gram of tissue was digested for 24 hours at 37°C and 65°C for the protease and basic digestive agents, respectively.

Digested samples were vacuum-filtered through a 1 μm pore-size polycarbonate filter (Whatman, Florham Park, NJ), which was dried and reweighed. The ratio of residual weight to initial tissue weight was compared for all 12 groups using ANOVA statistical analysis [Figure 1].

Results: Protease and basic digestion of porcine tissue were compared. The residual tissue weight after digestion was lowest (<1% init. wt.) after basic digestion with 5 M NaOH or 10 M KOH or 15 M KOH (p< 0.05, Figure 1). 5 M NaOH-digested samples were highly soluble; whereas other samples, including the 10 M and 15 M KOH retained small visible tissue clumps.

The three test conditions that yielded the lowest residual weight (5 M NaOH and 10 M KOH and 15 M KOH) were assessed for digestion of human capsule tissue. Capsule tissue was collected from 8 patients at the time of primary THR. One gram of human tissue was assigned to each concentration of basic digestive agent (18 g of pooled tissue were divided into 1 gram weights; for each treatment n=3). Digestion results for human hip tissue are shown in Figure 2. Digestion was highly effective with either 5 M NaOH or KOH over the range of basic digestive solutions (p< 0.05, Figure 2).

Discussion: This study is the first to demonstrate a wide range of efficiency for currently accepted methods of tissue digestion. We observed minimum residual tissue weight using 5 M NaOH solutions as compared to protease or basic digestive solutions. 5 M NaOH was also most effective for digestion of human hip tissue. These findings are an important comparison of existing approaches used for periprosthetic tissue digestion. This information will allow us to optimize and standardize the overall process of particle isolation.

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Table 1: Testing array for tissue digestion

<table>
<thead>
<tr>
<th>Digesting Agent</th>
<th>KOH</th>
<th>NaOH</th>
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<tbody>
<tr>
<td>5 M</td>
<td>2.81 g/10 mL</td>
<td>2.0 g/10 mL</td>
</tr>
<tr>
<td>10 M</td>
<td>5.60 g/10 mL</td>
<td>4.0 g/10 mL</td>
</tr>
<tr>
<td>15 M</td>
<td>8.40 g/10 mL</td>
<td>6.0 g/10 mL</td>
</tr>
</tbody>
</table>

Figure 1: Results showing % residual tissue weight following porcine hip tissue digestion and vacuum filtration. Digestion was most efficient for 5 M NaOH as compared to protease and other basic treatments. The decreased effectiveness of NaOH at higher molar concentrations has been attributed to the formation of sodium precipitates.

Figure 2: Results showing boxplots of % residual tissue weight following human hip capsular tissue digestion and vacuum filtration. Digestion was most efficient for 5 M NaOH or 5 M KOH as compared to other basic treatments. (p<0.05)