Identification of Optimal Tissue Digestion Methods for Polyethylene Wear Debris Analysis

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Introduction: Several methods have been proposed to isolate polyethylene wear particles from retrieved, digested tissue [1-3]. The first step in particle isolation entails digestion of the tissue sample using acid, base, or enzymes. However, few studies provide comparative assessments of particle isolation techniques, and existing standards (e.g., ASTM F561-05a) provide limited guidance on this topic. The purpose of this study was to identify an optimal digestion protocol to facilitate wear particle isolation from retrieved periprosthetic tissue.

Methods: Digestion methods using strong bases and enzymes were tested to compare their efficiency; acid digestion was ruled out because of induced changes in particle morphology [4]. Solutions of sodium hydroxide, potassium hydroxide (Fisher Scientific, Hampton, NH), Proteinase K powder (Bioline, Randolph, MA) and Liberase Blendzyme 3 powder (Roche Applied Science, (Indianapolis, IN) were prepared; each with three different concentrations. Porcine tissue samples were dissected from the hip joint. Samples (n=3) were randomly assigned to each test condition, as summarized in Table 1. Tissue samples were weighed and digested for 24 hours at 27° C and 65° C for the protein and basic digestive agents, respectively.

Molarity \ Digesting Agent	KOH	NaOH
5 M	2.81 g/10 mL	2.0 g/10 mL
10 M	5.60 g/10 mL	4.0 g/10 mL
15 M	8.40 g/10 mL	6.0 g/10 mL

Dose \ Digesting Agent	Proteinase K	Liberase Blendzyme 3
Low	10 mg/10 mL	1 mg/10 mL
Medium	20 mg/10 mL	2 mg/10 mL
High	30 mg/10 mL	3 mg/10 mL

Table 1: Testing array for tissue digestion.

Digested samples were vacuum-filtered through 1 mm pore-size polycarbonate filters (Whatman, Florham Park, NJ), which were then dried and reweighed. The ratio of unfiltered tissue to initial tissue weight was compared for all 12 test groups using analysis of variance.

The two test conditions yielding optimal results were retested to determine their effect on polyethylene particles. A weight of 0.02g of GUR 415 UHMWPE powder was added to 1g of porcine tissue and digested with 5M NaOH and 10M KOH solutions. Filters with digested and undigested particles were platinum sputter-coated and imaged under scanning electron microscopy with a beam intensity of 3kV and magnification of 5000x (XL-30 Environmental SEM, Drexel University).

Results: Statistically, percentage values for 5M NaOH and 10M KOH showed the least filtered tissue residual ($p < 0.05$, Figure 1). Residual tissue represented only 0.33% and 0.49% of the initial weight for 5M NaOH- and 10M KOH-digested samples, respectively. Tissue digested with NaOH was more soluble; KOH-digested samples and others retained small visible tissue clumps. SEM micrographs of digested and undigested PE powder show retention of sub-micron fibrils (Figure 2).

Discussion: We observed shortened tissue digestion times and retention of small-scale particle morphology using 5M NaOH solution over a wide range of alternate solution candidates. These data will be useful in the further development of standard test methods. Human tissue is believed to behave similarly for these digestion methods; additional research is underway to confirm this hypothesis.

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References: [1] Tipper JL. Trans Orth Res Soc. 1997;29:355. [2] Campbell P. J Biomed Mater Res. 1995;29:127–31. [3] Visentin. Biomaterials. 2004;25: 5531-7. [4] Niedzwiecki S. J Biomed Mater Res. 2001;56:245–249.

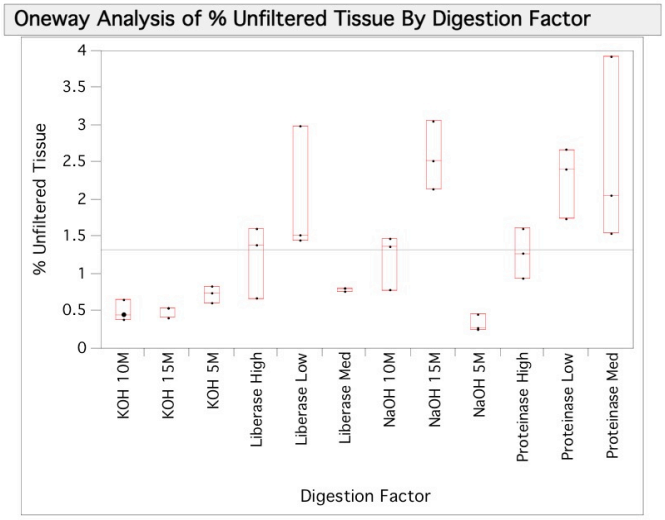

Figure 1: % Unfiltered Tissue by Digestion Factor.

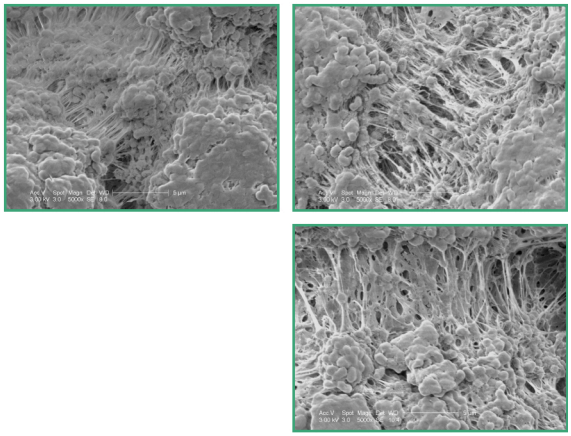

Figure 2: SEM micrographs showing intact, submicron fibrils. Before digestion (top left), 10 M KOH digestion (top right), 5M NaOH digestion (bottom right). (5000x magnification)