Published in final edited form as:

J Biomed Mater Res B Appl Biomater. 2009 October ; 91(1): 409–418. doi:10.1002/jbm.b.31416.

Comparison of Periprosthetic Tissue Digestion Methods for Ultra-High Molecular Weight Polyethylene Wear Debris Extraction

R.M. Baxter¹, M.J. Steinbeck¹, J.L. Tipper², J. Parvizi³, M. Marcolongo⁴, and S.M. Kurtz^{1,5}

¹ Department of Biomedical Engineering, Drexel University, Philadelphia, PA 19104

² Institute of Medical & Biological Engineering, University of Leeds, Leeds, LS2 9JT United Kingdom

³ The Rothman Institute of Orthopedics at Thomas Jefferson University, Philadelphia, PA 19107

⁴ Department of Materials Science and Engineering, Drexel University, Philadelphia, PA 19104

⁵ Exponent Inc, Philadelphia, Pennsylvania 19104

Abstract

There is considerable interest in characterization of wear debris from polyethylene (UHMWPE) bearing components used in total joint replacement. To isolate UHMWPE wear debris, tissue samples must be excised from regions adjacent to revised UHMWPE implant components, followed by exposure to one of many available tissue digestion methods. Numerous studies demonstrate successful digestion, but the relative efficiency of each method is not clear. The purpose of this study was to evaluate a variety of conditions for tissue digestion to provide a quantitative comparison of methods. Porcine and human hip tissues were exposed for 24 hr to basic, acidic or enzymatic agents, filtered and digestion efficiency calculated based on the percentage of initial to final tissue weight. Of the conditions tested, 5M NaOH, 5M KOH, 15M KOH or 15.8M HNO₃ yielded the most complete porcine hip tissue digestion (<1% residual tissue weight; $p < 0.05$). Proteinase K and Liberase Blendzyme 3 did not effectively digest tissue in a 24 hour period. Similar to results from the porcine dataset, human tissues digestion was most efficient using 5M NaOH, 5M KOH or 15.8M HNO₃ (<1% residual tissue weight; $p < 0.05$). To verify that particle surface modifications did not occur after prolonged reagent exposure, GUR415 and Ceridust 3715 particles were immersed in each solution for 24 hours. Overall, this study provides a framework for thorough and efficient digestive methods for UHMWPE wear debris extraction.

Keywords

ultra-high molecular weight polyethylene; wear debris; periprosthetic tissue; tissue digestion

Introduction

UHMWPE-mediated osteolysis is a significant complication of total joint replacement, which contributes to decreased implant fixation and limits the long-term survival of joint replacement components. 1–2 UHMWPE wear debris generated by articulating implant component surfaces is implicated in the activation of a biological cascade associated with the onset of osteolysis and, ultimately, aseptic implant loosening. 3–6 Specifically, particle-

induced macrophage activation marks the beginning of a chronic inflammatory response and the release of various cytokines and factors that promote adverse biological responses and/or bone loss. The formation of osteoclasts, bone resorbing cells, as well as a partial reduction in bone formation, leads to a predominance of bone loss at the prosthesis-bone interface.⁷⁻⁹ The UHMWPE wear debris-induced activation and progression of chronic inflammation, and the potential for osteolysis, is dependent on several aspects of particle generation, which include particle accumulation, size and shape.¹⁰⁻²⁰

Particulate wear debris results from the motion between opposing implant bearing surfaces. The generation of wear debris is a function of several variables, which include lubrication, sliding distance, specifics of the manufacturing of the UHMWPE component and amount of use of the joint.²¹ Shanbhag et al. (2000) showed that wear particle size also varies by joint type based on differences in the loading and conformity of mating bearing surfaces.¹⁴ In addition, implant exposure to oxygen-rich biological fluid can cause *in vivo* degradation of the mechanical properties of UHMWPE components, which leads to an increase in the overall wear rate.^{21,22} As new polymer bearing components are introduced, such as highly cross-linked UHMWPE, a detailed understanding of the UHMWPE wear behavior is necessary.

The process of studying clinically relevant wear debris begins at the time of implant removal and the surgical excision of adjacent or periprosthetic tissue. This is followed by tissue digestion to extract embedded UHMWPE, metal, ceramic and/or bone cement debris. Previous tissue digestive approaches to isolate UHMWPE wear debris include the use of basic, acidic and enzymatic reagent solutions (Table 1). In general, periprosthetic tissue digestion has been accomplished using a range of reagents, temperatures, solution concentrations, and digestive time periods. Sodium hydroxide (NaOH) and potassium hydroxide (KOH) are common basic reagents used for tissue digestion. Campbell et al. (1995) digested tissue at 65°C for 1–5 hours with 5M NaOH, and these conditions have been subsequently widely used by others.^{16,23,24} The digestion time was extended to 24 hours by other investigators to achieve complete digestion of formalin fixed tissues.^{25,26} Shanbhag et al. (1994) used 4M KOH at 56°C for 48 hours to digest formalin fixed tissue, whereas Tipper et al. (2000) and Howling et al. (2001) used 12M KOH at 60°C for 2–5 days.²⁷⁻²⁹ Tissue digestion to purify and extract UHMWPE wear debris has also been achieved using acid reagent solutions. Margevicius et al. (1994) digested formalin fixed tissue at room temperature for 48 hours using concentrated nitric acid (HNO₃), which were conditions used by others to digest fresh tissue.³⁰⁻³³ Hydrochloric acid (HCl) has been used in limited instances, but with poor efficiency compared to tissue digestion in concentrated HNO₃.³⁰ Another approach has been to use enzymatic solutions to digest tissue. Maloney et al. (1995) exposed tissue to 7mg/ml of papain in phosphate buffer (pH 6.5) containing 2mM N-acetylcysteine at 65°C, and other investigators used 0.3mg/ml of papain.³⁴⁻³⁷ The use of papain required 2–3 days and daily addition of fresh enzyme stock. In another study, tissue was digested in 2mg/ml of collagenase in phosphate buffer (pH 7.0) for 24 hours at 37°C.³⁸ While each of these approaches have been used to isolate wear debris, the relative digestive efficiency of these agents remains unclear.

Based on many of the previous studies, the ASTM standard document 39 for particle analysis suggests a number of digestion solutions can be used to extract UHMWPE particles. However, these standards provide neither conclusive guidance nor comparative assessment of particle isolation techniques. The purpose of this study was to evaluate a variety of conditions for tissue digestion to provide a quantitative and comparative assessment of digestion efficiency.

Materials and Methods

Materials

The following materials were used: sodium hydroxide (NaOH, Catalog #: S3181), potassium hydroxide (KOH, Catalog #: 1310-58-3) (Fisher Scientific, Hampton, NH), Proteinase K (Catalog #: BIO-37037, Biotline, Randolph, MA) Liberase Blendzyme 3 (Catalog #: 1814184, Roche Applied Science, (Indianapolis, IN), nitric acid (HNO₃ Catalog# A483-212) and NucleoporeR polycarbonate filters with a pore size of 1 mm (Catalog #: 110410, Whatman, Florham Park, NJ). Proteinase K is a serine protease that cleaves peptide bonds in proteins; Liberase Blendzyme 3 contains collagenase and neutral protease to cleave both collagen and peptide bonds in proteins.

Tissue Collection

Porcine hip tissue was obtained from six regions of a single animal cadaver. Human hip capsule tissue was collected from eight patients at the time of primary total hip replacement surgery. All tissue samples were wrapped in saline-wetted gauze, transported to the laboratory in a sterile container on wet ice, flash frozen and stored at -80°C until the time of testing. Tissue collection and storage procedures were in accordance with the IRB guidelines of each participating institutions.

Tissue Digestion Conditions

Tissue samples were cut into one-gram tissue pieces, which were then cut into 0.25 x 0.25 cm cubes and exposed for 24 hr to basic, acidic or enzymatic solutions to compare digestion efficiency. The tissues were processed by a single researcher, and all efforts were made to ensure the resultant size of each tissue was consistent by using a 0.25 cm grid. The digestion agents were prepared in 10 ml aliquots of distilled water for bases and acids, and PBS pH 7.4 for enzymes. After 24 hours, each tissue digestive solution combination was diluted to a total volume of 200 ml by addition of distilled water. Dilution was necessary to avoid exposure of the polycarbonate filter to strong alkalinity (> 6M NaOH), which can compromise the structural integrity of the filter. NucleoporeR polycarbonate filters with a pore size of 1 mm were weighed prior to sample filtration. Diluted samples were vacuum-filtered, transferred to Petri dishes and thoroughly dried for one hour by convection heat lamps followed by four to six hours at ambient temperature. Filters were reweighed and weight measurements were recorded. All samples were weighed on a calibrated scale with precision to four decimal places.

To compare the efficacy of basic, enzymatic, and acidic digestion methods, porcine or human hip tissues were subjected to several different agents. For basic digestion, three concentrations of NaOH and KOH were assigned to create a 2x3 array with six test conditions (porcine, Table 2; human, Table 3). The concentrations for each base were 5, 10 and 15M. Each test condition was repeated six times.

For enzymatic digestion, Proteinase K and Liberase Blendzyme 3 were chosen for porcine tissue digestion. Three protease concentrations were assigned to a 2x3 array with six total test conditions (Table 2). The concentrations for Proteinase K were 1, 2 and 3 mg/ml; concentrations for Liberase Blendzyme 3 were 0.1, 0.2 and 0.3 mg/ml. Enzyme concentrations were selected based on amounts of proteinase and collagenase used in previous wear debris isolation studies [25, 26]. Each test condition was repeated six times.

For acid digestion, two concentrations of HNO₃ were used to digest tissue. The concentrations for each acid digestion were 11M (~49%) and 15.8M (concentrated, 70%) (porcine, Table 2; human, Table 3). Each test condition was repeated three times.

For all treatments, one gram of porcine or human hip tissue was digested in an oscillating water bath for 24 hours at 65°C, 37°C, or 25°C for basic, enzymatic, or acidic digestion, respectively. No chloroform/methanol extraction was performed prior to digestion.

Tissue Digestion Efficiency Calculations

To evaluate digestion efficiency, initial and final filter and tissue weights were measured, and the percentage of residual or undigested tissue was determined for each test condition. The final filter weights represented the amount of residual digested tissue that did not pass through the 1 mm filter pores during vacuum-filtration.

ESEM

To check for surface modifications, GUR415 UHMWPE powder and Ceridust 3715 UHMWPE particles were exposed to optimal digestive conditions, 5M NaOH, 5M KOH and 15.8M HNO₃. Filtered samples were mounted on aluminum ESEM stubs with double-sided adhesive tape. Using a 208HR High Resolution Cressington Sputter Coater, filtered particulate samples were coated with Pt-Pd for 10 seconds to yield an appropriate coating thickness of 4 nanometers. Samples were imaged at 3500x or 5000x magnification with a beam intensity of 3kV using an Environmental Scanning Electron Microscope at Drexel University's Centralized Research Facility (Philips, XL-30).

Statistical Analysis

Statistical analysis was performed in JMP 7.0 (SAS, Cary, NC) using one-way ANOVA with a significance level of $p < 0.05$. Figures were plotted in KaliedaGraph 3.6 (Synergy Software, Reading, PA). A post-hoc analysis (JMP 7.0, SAS, Cary, NC) was used to calculate the power associated with the comparison between different concentrations and digestive methods. The statistical power was 0.970 for the porcine tissue, 0.830 for the formalin-fixed tissue and 0.977 for the human tissue digestions, which is greater than the usual standard of >0.8 for sufficiently powered analyses.

Results

Porcine Tissue Digestion Comparisons

For porcine hip tissue, results of each digestion condition are shown in Figure 1. The residual tissue percentages after 24 hours of digestion were statistically lowest for 5M NaOH, 5M KOH and 15M KOH ($p < 0.05$). These treatments resulted in final tissue weights of $< 1\%$ of the initial tissue weight. However, based on photodocumentation (Fig. 2), only filters from the 5M NaOH digested samples showed minimal amounts of undigested tissue material. All other samples, including the 5M, 10M and 15M KOH treated samples, contained undigested cellular material. Filter images in figure 2 are representative of the amount of residual tissue debris.

Porcine tissue digested with enzymatic solutions yielded varied results after 24 hours at 37°C. Tissue samples were digested to a very limited extent by all concentrations of Proteinase K, and most of the original tissue pieces were still visible after 24 hours (Figs. 1, 2). Moderate improvements in digestion efficiency were observed with increased enzyme concentration (3mg/ml), although the improvements were not statistically significant. For Liberase Blendzyme 3 improved levels of tissue digestion were observed compared to Proteinase K, but the digestion did not reach the lowest residual tissue weight of $< 1\%$ (Figs. 1, 2).

Porcine tissue digested in 15.8M HNO₃ resulted in very thorough digestion ($p < 0.05$; Fig. 1). The residual tissue weight was less than 1% of the initial tissue weight, and little or no cellular material was observed on the filter (Fig. 2). Tissue digestion with 11M HNO₃ (50%

v/v) was not as complete, and residual tissue clumps and the filter itself, had a yellowed appearance.

Formalin Fixed Tissue Digestion

To evaluate fixed tissue digestion, porcine tissue was placed in formalin fixative (10% (v/v) neutral buffered formalin) for two weeks. One gram of porcine tissue was cut into small pieces (0.25 x 0.25 cm) and placed into either 5M NaOH, 5M KOH, or 15.8M HNO₃ for 24 hours. Digested samples were filtered and the percentage of undigested tissue was calculated based on the original and final filter plus tissue weights. Formalin-fixed tissue samples yielded higher residual weights when digested using 5M NaOH or 5M KOH compared to non-fixed tissue ($p < 0.05$; Fig. 3). Whereas, digestion of formalin-fixed tissues was highly efficient using HNO₃, resulting in less than 1% of the original tissue weight (Fig. 3). A finding that was essentially identical to the results for non-fixed tissue digestion. The overall percentage of residual tissue weight was statistically lower for HNO₃ digestion of formalin-fixed tissue as compared to basic digestion ($p < 0.05$; Fig. 3). Representative images of formalin-fixed porcine tissue digests following vacuum filtration are shown in figure 4.

Human Tissue Digestion Comparisons

Based on the most efficient digestion of porcine tissues, human capsule tissue samples were exposed to basic and acidic digestion using conditions outlined in Table 3. Digestion was highly effective with 5M NaOH, 5M KOH or 15.8M HNO₃ when compared to other concentrations ($p < 0.05$; Fig. 5). These treatments resulted in final tissue weights of less than 1% of the initial tissue weight. Digestion results for higher concentrations of NaOH and KOH were more variable; specifically for NaOH, higher molar concentrations did not effectively digest human capsule tissue after 24 hours.

ESEM Evaluation of Particle Surfaces

To verify that basic and acidic solutions did not alter the surface of UHMWPE particles, GUR415 UHMWPE powder and Ceridust 3715 particles were separately immersed in 5M NaOH, 5M KOH, or 15.8M HNO₃ for 24 hours. GUR 415 UHMWPE powder and Ceridust 3715 particles were also immersed in distilled water for 24 hours as a control. GUR 415 was selected to be representative of a conventional UHMWPE implant component (> 10 mm), whereas Ceridust 3715 was selected as a more clinically relevant supply of 5–10 mm-diameter HDPE particles. Recently, Ceridust particles have also been used on exposed mouse calvaria during an in vivo study of particle biological activity.⁴⁰ Treated particles and the untreated control group were filtered onto 1 mm polycarbonate filters, and dried overnight at ambient temperature in a Petri dish. After preparation for ESEM, the samples were imaged with a beam intensity of 3kV to avoid beam-induced melting of the UHMWPE. The representative images presented in Figure 6 show that exposure of GUR415 UHMWPE powder to 5M NaOH, 5M KOH, or 15.8M HNO₃ did not result in changes to the particle surface. Specifically, the treated particles retained the small fibril-like features that were visible on the untreated control group. Inspection of Ceridust particles from each of the digestive and control conditions revealed a similar appearance of the particle perimeter/boundary (Fig. 7). Both results verify that exposure to basic or acidic digestive solutions for a period of 24 hours does not etch or alter the UHMWPE surface.

Discussion

This study undertook a quantitative comparison of existing methods used to digest periprosthetic tissue for UHMWPE wear debris analysis. First, a method for evaluating and comparing the efficiency of tissue digestion solutions by quantifying residual tissue weight was reported. Second, human tissues were most completely digested by 5M NaOH, 5M

KOH or 15.8M HNO₃ solutions. All other concentrations of the acid and base digestive agents, as well as enzymatic solutions, contained undigested pieces of tissue. Third, ESEM evaluation verified that UHMWPE particles and Ceridust particles exposed to NaOH, KOH and HNO₃ solutions do not undergo morphological changes. Finally, digestion of formalin-fixed tissue was most complete with 15.8M HNO₃.

The current study results show that, over a period of 24 hours, neither Proteinase K nor Liberase Blendzyme 3 effectively digested tissue. However successful enzymatic tissue digestion has been reported by a number of investigators (Table 1). Campbell et al. (1994) reported that successful digestion was achieved using bacterial collagenase over a 24-hour period, which is the shortest enzymatic method presented in the literature. 38 Successful digestion was based on a visual assessment of the digest after ultracentrifugation. Other researchers have used papain, which required the daily addition of fresh enzyme over 3 days to extract particles. 34–37 In the present study, digestion using Liberase Blendzyme 3 and Proteinase K concentrations was not extended beyond 24 hrs, since the need for constant monitoring and extended time involvement as well as the high cost of materials make this approach much less appealing.

Similar to other studies, lower molar solutions of NaOH or KOH were found to be effective digestive solutions. Studies by Campbell et al. (1995) showed that 5M NaOH effectively solubilized tissue within a 24 hour time period. 16·23·24 Shanbhag et al. (1994) used 4M KOH at 56°C for 48 hr to digest tissue. 28 Additionally, concentrated HNO₃ was an effective digestion solution. This is in agreement with Slouf et al. (2007) who used concentrated HNO₃ over a 48 hour time period. 30·32 Overall, the present study shows that using 5M NaOH or KOH at 65°C or 15.8M HNO₃ at room temperature resulted in ≥99% of tissue digestion after 24 hours.

Since many of the periprosthetic tissues are placed in formalin, the current study evaluated digestion efficiency of the acid and base solutions on formalin-fixed porcine tissues. Digestion using concentrated HNO₃ was significantly more efficient than 5M NaOH or KOH solutions. Unlike the basic solutions, the digestive action of 15.8M HNO₃ was not affected by formalin fixation as compared with non-fixed tissues (Figs. 3, 4). Margevicius et al. (1994) and Hirakawa et al. (1996) used concentrated nitric acid for 48+ hours to digest formalin-fixed tissue. 30 The current finding that formalin fixative decreases digestion efficiency is in agreement with methods employed by Hahn et al. (1997) and Wolfarth et al. (1997) who used 5M NaOH for 24hr, and by Tipper et al. (2000) and Howling et al. (2001) who used 12M KOH for 2–5 days to achieve complete digestion. 27·29 The authors do not wish to suggest formalin fixation as a general standard for collecting tissue retrieval, as this method of preservation can impose limits on complementary analyses of tissue samples by histological or immunohistochemical methods. However, this data suggests that thorough tissue digestion can be achieved using concentrated HNO₃ for instances where formalin fixation is the existing protocol of tissue collection.

The results of the current quantitative study highlight the variability of current tissue digestion methods. Of the fourteen conditions tested, three digestive solutions resulted in residual tissue weight less than 1% of the initial sample tissue weight: 5M NaOH, 5M KOH and 15.8M HNO₃. Several advantages of using HNO₃ are that sample dilution and thus the final sample volume can be reduced because concentrated HNO₃ does not affect the polycarbonate filter used to filter out the UHMWPE wear debris, and it does not effect the UHMWPE particle morphology. Additionally, concentrated nitric acid has been used to isolate metal particles resulting in <1% of particle dissolution and no change in morphology after 48 hours, as confirmed by atomic absorption spectroscopy and ESEM, respectively. 30 In contrast, alkali solutions ranging from 2–2M have been shown to affect the size and ion

composition of metal particles as early as 2 hours after exposure. 41-42 Based on the current evaluation of digestion efficiency, and previous wear debris analysis, concentrated HNO₃ is both effective and the least destructive digestive solution. These results provide a basic framework for expedited and efficient tissue digestion to extract UHMWPE or metal wear debris.

Acknowledgments

Supported by NIH R01 AR47904

References

1. Jasty M, Rubash HE, Muratoglu O. Highly cross-linked polyethylene: the debate is over--in the affirmative. *J Arthroplasty*. 2005; 20(4 Suppl 2):55–8. [PubMed: 15991131]
2. McKellop H, Shen FW, Lu B, Campbell P, Salovey R. Development of an extremely wear-resistant ultra high molecular weight polyethylene for total hip replacements. *J Orthop Res*. 1999; 17(2):157–67. [PubMed: 10221831]
3. Agarwal S. Osteolysis: Science, incidence, and diagnosis. *Curr Orthop June*. 2004; 18(3):220–231.
4. Amstutz HC, Campbell P, Kossovsky N, Clarke IC. Mechanism and clinical significance of wear debris-induced osteolysis. *Clin Orthop Relat Res*. 1992; (276):7–18. [PubMed: 1537177]
5. Revell PA, al-Saffar N, Kobayashi A. Biological reaction to debris in relation to joint prostheses. *Proc Inst Mech Eng [H]*. 1997; 211(2):187–97.
6. Willert HG. Reactions of the articular capsule to wear products of artificial joint prostheses. *J Biomed Mater Res*. 1977; 11(2):157–64. [PubMed: 140168]
7. Purdue PE, Koulouvaris P, Nestor BJ, Sculco TP. The central role of wear debris in periprosthetic osteolysis. *Hss J*. 2006; 2(2):102–13. [PubMed: 18751821]
8. Sabokbar A, Fujikawa Y, Neale S, Murray DW, Athanasou NA. Human arthroplasty derived macrophages differentiate into osteoclastic bone resorbing cells. *Ann Rheum Dis*. 1997; 56(7):414–20. [PubMed: 9486003]
9. Greenfield EM, Bi Y, Ragab AA, Goldberg VM, Van De Motter RR. The role of osteoclast differentiation in aseptic loosening. *J Orthop Res*. 2002; 20(1):1–8. [PubMed: 11855378]
10. Green TR, Fisher J, Stone M, Wroblewski BM, Ingham E. Polyethylene particles of a 'critical size' are necessary for the induction of cytokines by macrophages in vitro. *Biomaterials*. 1998; 19(24):2297–302. [PubMed: 9884043]
11. Green TR, Fisher J, Matthews JB, Stone MH, Ingham E. Effect of size and dose on bone resorption activity of macrophages by in vitro clinically relevant ultra high molecular weight polyethylene particles. *J Biomed Mater Res*. 2000; 53(5):490–7. [PubMed: 10984696]
12. Schmalzried TP, Campbell P, Schmitt AK, Brown IC, Amstutz HC. Shapes and dimensional characteristics of polyethylene wear particles generated in vivo by total knee replacements compared to total hip replacements. *J Biomed Mater Res*. 1997; 38(3):203–10. [PubMed: 9283965]
13. Tipper JL, Galvin AL, Williams S, McEwen HM, Stone MH, Ingham E, Fisher J. Isolation and characterization of UHMWPE wear particles down to ten nanometers in size from in vitro hip and knee joint simulators. *J Biomed Mater Res A*. 2006; 78(3):473–80. [PubMed: 16721797]
14. Shanbhag AS, Bailey HO, Hwang DS, Cha CW, Eror NG, Rubash HE. Quantitative analysis of ultrahigh molecular weight polyethylene (UHMWPE) wear debris associated with total knee replacements. *J Biomed Mater Res*. 2000; 53(1):100–10. [PubMed: 10634959]
15. Kadoya Y, Revell PA, Kobayashi A, al-Saffar N, Scott G, Freeman MA. Wear particulate species and bone loss in failed total joint arthroplasties. *Clin Orthop Relat Res*. 1997; (340):118–29. [PubMed: 9224247]
16. Kobayashi A, Bonfield W, Kadoya Y, Yamac T, Freeman MA, Scott G, Revell PA. The size and shape of particulate polyethylene wear debris in total joint replacements. *Proc Inst Mech Eng [H]*. 1997; 211(1):11–5.

17. Yang SY, Ren W, Park Y, Sieving A, Hsu S, Nasser S, Wooley PH. Diverse cellular and apoptotic responses to variant shapes of UHMWPE particles in a murine model of inflammation. *Biomaterials*. 2002; 23(17):3535–43. [PubMed: 12109677]
18. Ren W, Yang SY, Fang HW, Hsu S, Wooley PH. Distinct gene expression of receptor activator of nuclear factor-kappaB and rank ligand in the inflammatory response to variant morphologies of UHMWPE particles. *Biomaterials*. 2003; 24(26):4819–26. [PubMed: 14530078]
19. Shanbhag AS, Jacobs JJ, Black J, Galante JO, Glant TT. Macrophage/particle interactions: effect of size, composition and surface area. *J Biomed Mater Res*. 1994; 28(1):81–90. [PubMed: 8126033]
20. Goodman SB, Davidson JA, Song Y, Martial N, Fornasier VL. Histomorphological reaction of bone to different concentrations of phagocytosable particles of high-density polyethylene and Ti-6Al-4V alloy in vivo. *Biomaterials*. 1996; 17(20):1943–7. [PubMed: 8894085]
21. Schmalzried TP, Callaghan JJ. Wear in total hip and knee replacements. *J Bone Joint Surg Am*. 1999; 81(1):115–36. [PubMed: 9973062]
22. Kurtz SM, Rinnac CM, Hozack WJ, Turner J, Marcolongo M, Goldberg VM, Kraay MJ, Edidin AA. In vivo degradation of polyethylene liners after gamma sterilization in air. *J Bone Joint Surg Am*. 2005; 87(4):815–23. [PubMed: 15805212]
23. Campbell P, Ma S, Yeom B, McKellop H, Schmalzried TP, Amstutz HC. Isolation of predominantly submicron-sized UHMWPE wear particles from periprosthetic tissues. *J Biomed Mater Res*. 1995; 29(1):127–31. [PubMed: 7713952]
24. Mabrey JD, Afsar-Keshmiri A, Engh GA, Sychterz CJ, Wirth MA, Rockwood CA, Agrawal CM. Standardized analysis of UHMWPE wear particles from failed total joint arthroplasties. *J Biomed Mater Res*. 2002; 63(5):475–83. [PubMed: 12209890]
25. Hahn DW, Wolfarth DL, Parks NL. Analysis of polyethylene wear debris using micro-Raman spectroscopy: a report on the presence of beta-carotene. *J Biomed Mater Res*. 1997; 35(1):31–7. [PubMed: 9104696]
26. Wolfarth DL, Han DW, Bushar G, Parks NL. Separation and characterization of polyethylene wear debris from synovial fluid and tissue samples of revised knee replacements. *J Biomed Mater Res*. 1997; 34(1):57–61. [PubMed: 8978653]
27. Tipper JL, Ingham E, Hailey JL, Besong AA, Fisher J, Wroblewski BM, Stone MH. Quantitative analysis of polyethylene wear debris, wear rate and head damage in retrieved Charnley hip prostheses. *J Mater Sci Mater Med*. 2000; 11(2):117–24. [PubMed: 15348056]
28. Shanbhag AS, Jacobs JJ, Glant TT, Gilbert JL, Black J, Galante JO. Composition and morphology of wear debris in failed uncemented total hip replacement. *J Bone Joint Surg Br*. 1994; 76(1):60–7. [PubMed: 8300684]
29. Howling GI, Barnett PI, Tipper JL, Stone MH, Fisher J, Ingham E. Quantitative characterization of polyethylene debris isolated from periprosthetic tissue in early failure knee implants and early and late failure Charnley hip implants. *J Biomed Mater Res*. 2001; 58(4):415–20. [PubMed: 11410900]
30. Margevicius KJ, Bauer TW, McMahon JT, Brown SA, Merritt K. Isolation and characterization of debris in membranes around total joint prostheses. *J Bone Joint Surg Am*. 1994; 76(11):1664–75. [PubMed: 7962027]
31. Slouf M, Eklova S, Kumstatova J, Berger S, Synkova H, Sosana A, et al. Isolation, characterization, and quantification of polyethylene wear debris from periprosthetic tissues around total joint replacements. *Wear*. April; 2007 262(9–10):1171–81.
32. Hallab NJ, Cunningham BW, Jacobs JJ. Spinal implant debris-induced osteolysis. *Spine*. 2003; 28(20):S125–38. [PubMed: 14560184]
33. Hirakawa K, Bauer TW, Stulberg BN, Wilde AH, Secic M. Characterization and comparison of wear debris from failed total hip implants of different types. *J Bone Joint Surg Am*. 1996; 78(8):1235–43. [PubMed: 8753716]
34. Maloney WJ, Smith RL, Schmalzried TP, Chiba J, Huene D, Rubash H. Isolation and characterization of wear particles generated in patients who have had failure of a hip arthroplasty without cement. *J Bone Joint Surg Am*. 1995; 77(9):1301–10. [PubMed: 7673277]

35. Dean DD, Schwartz Z, Liu Y, Blanchard CR, Agrawal CM, Mabrey JD, Sylvia VL, Lohmann CH, Boyan BD. The effect of ultra-high molecular weight polyethylene wear debris on MG63 osteosarcoma cells in vitro. *J Bone Joint Surg Am.* 1999; 81(4):452–61. [PubMed: 10225790]
36. Wirth MA, Agrawal CM, Mabrey JD, Dean DD, Blanchard CR, Miller MA, Rockwood CA Jr. Isolation and characterization of polyethylene wear debris associated with osteolysis following total shoulder arthroplasty. *J Bone Joint Surg Am.* 1999; 81(1):29–37. [PubMed: 9973051]
37. Koseki H, Matsumoto T, Ito S, Doukawa H, Enomoto H, Shindo H. Analysis of polyethylene particles isolated from periprosthetic tissue of loosened hip arthroplasty and comparison with radiographic appearance. *J Orthop Sci.* 2005; 10(3):284–90. [PubMed: 15928891]
38. Campbell P, Ma S, Schmalzried T, Amstutz HC. Tissue digestion for wear debris particle isolation. *J Biomed Mater Res.* 1994; 28(4):523–6. [PubMed: 8006056]
39. ASTM F561. Standard Practice for Retrieval and Analysis of Medical Devices, and Associated Tissues and Fluids. ASTM International; West Conshohocken, PA: 2005a. www.astm.org
40. Illgen RL 2nd, Bauer LM, Hotujec BT, Kolpin SE, Bakhtiar A, Forsythe TM. Highly crosslinked vs conventional polyethylene particles: relative in vivo inflammatory response. *J Arthroplasty.* 2009; 24(1):117–24. [PubMed: 18534436]
41. Catelas I, Bobyn JD, Medley JB, Krygier JJ, Zukor DJ, Petit A, Huk OL. Effects of digestion protocols on the isolation and characterization of metal-metal wear particles. I. Analysis of particle size and shape. *J Biomed Mater Res.* 2001; 55(3):320–9. [PubMed: 11255185]
42. Catelas I, Bobyn JD, Medley JJ, Zukor DJ, Petit A, Huk OL. Effects of digestion protocols on the isolation and characterization of metal-metal wear particles. II. Analysis of ion release and particle composition. *J Biomed Mater Res.* 2001; 55(3):330–7. [PubMed: 11255186]

Figure 1. Boxplot representation of percent residual tissue weight following porcine hip tissue digestion and vacuum filtration

Digestion results were statistically significant for 5M NaOH, 5M KOH, 15M KOH (n=6) and 15.8M HNO₃ (n=3) solutions, as compared to enzymatic and other solutions (p<0.05). Following digestion with 5M NaOH and 15.8M HNO₃, no residual tissue clumps were observed. The decreased effectiveness of NaOH at higher molar concentrations may be attributed to the formation of sodium precipitates.

Figure 2. Representative images of porcine tissue digests following vacuum filtration
Residual tissue was not observed on filters from tissues digested in 5M NaOH and 15.8M HNO₃.

Figure 3. Boxplot representation of percent residual tissue weight following formalin-fixed porcine hip tissue digestion and vacuum filtration

Digestion of formalin-fixed porcine tissue samples was most complete in 15.8M HNO₃, whereas 5M NaOH and 5M KOH had statistically higher amounts of residual tissue weight following vacuum filtration (n=3, p<0.05). A comparison of the unfixed tissue and the formalin-fixed tissue results indicated that only digestion in 15.8M HNO₃ was unaffected by fixation.

Figure 4. Representative images of formalin-fixed porcine tissue digests following vacuum filtration

Residual tissue was not observed on filters from tissues digested in 15.8M HNO₃.

Figure 5. Boxplot representation of percent residual tissue weight following human hip capsule tissue digestion and vacuum filtration

Digestion was most efficient for 5M NaOH 5M KOH or 15.8M HNO₃ solutions, as compared to other treatments, < 1% residual weight; (n = 3; p<0.05).

Figure 6. Environmental Scanning Electron Microscopy (ESEM) of GUR415 PE particles exposed to digestive solutions. GUR415 PE particles retain submicron features following immersion in digestive solutions

(A) ESEM micrograph of a control particle not subjected to digestion. Particles do not show surface changes following immersion in (B) 5M NaOH, (C) 5M KOH, (D) 15.8M HNO₃ for 24 hours.

Figure 7. Environmental Scanning Electron Microscopy (ESEM) of Ceridust PE particles exposed to digestive solutions

(A) ESEM micrograph of control Ceridust 3715 particles not subjected to digestion. Particles do not show surface changes following immersion in (B) 5M NaOH, (C) 5M KOH, (D) 15.8M HNO₃ for 24 hours.

Table 1

Summary of tissue digestion approaches to isolate UHMWPE wear debris

Reference	Year	Digestion Method	Timing	Temperature	Tissue Specifics
Campbell (#23)	1995	5M NaOH	1–5 hours	65°C	Fresh
Kobayashi (#16)	1997	5M NaOH	1–5 hours	65°C	Fresh
Mabrey (#24)	2002	5M NaOH	1–5 hours	65°C	Fresh
Hahn (#25)	1997	5M NaOH	24 hours	65°C	Stored in 10% v/v formalin
Wolfarth (#26)	1997	5M NaOH	24 hours	65°C	Stored in 10% v/v formalin
Shanbhag (#27)	1994	4M KOH	48 hours	56°C	Fresh
Tipper (#28)	2000	12M KOH	2–5 days	65°C	Stored in 10% v/v formalin
Howling (#29)	2001	12M KOH	2–5 days	60°C	Stored in 10% v/v formalin
Margevicius (#30)	1994	70% HNO3	24 hours	Room temp.	Stored in 10% v/v formalin
Slouf (#31)	2004	70% HNO3	24 hours	Room temp.	Freeze-dried prior to digestion
Hirakawa (#32)	1996	70% HNO3	24 hrs + 48 hrs	Room temp.	Stored in 10% v/v formalin
Maloney (#33)	1995	Papain 7 mg/ml 0.05M PBS, pH 6.5, 2mM NAC. Fresh papain each day	2 days	65°C	Stored in 10% v/v formalin
Dean (#34)	1999	Papain 0.3mg/ml 0.05M PBS, pH 6.5, 2mM NAC. Fresh papain each day	3 days	65°C	Stored in 10% v/v formalin
Wirth (#35)	1999	Papain 0.3mg/ml 0.05M PBS, pH 6.5, 2mM NAC. Fresh papain each day	3 days	65°C	Stored in 10% v/v formalin
Koseki (#36)	2005	Papain 0.3mg/ml 0.05M PBS, pH 6.5, 2mM NAC. Fresh papain each day	3 days	65°C	Stored in 10% v/v formalin
Campbell (#37)	1994	Collagenase 2mg/ml 0.1M phosphate buffer, pH 7.0	24 hours	37°C	Fresh

Table 2

Solutions and concentrations used for digestion of porcine hip tissue

PORCINE TISSUE DIGESTION	Concentration		
	5M	10M	15M
NaOH	5M	10M	15M
KOH	5M	10M	15M
Proteinase K	1mg/ml	2mg/ml	3mg/ml
Liberase Blendzyme 3	0.1mg/ml	0.2mg/ml	0.3mg/ml
HNO ₃	---	11M	15.8M

Table 3

Solutions and concentrations used for digestion of human hip capsule tissue

HUMAN HIP CAPSULE DIGESTION	Concentration		
NaOH	5M	10M	15M
KOH	5M	10M	15M
HNO ₃	---	11M	15.8M