Designation: F561 – 13

Standard Practice for Retrieval and Analysis of Medical Devices, and Associated Tissues and Fluids[1]

This standard is issued under the fixed designation F561; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 This practice covers recommendations for the retrieval, handling, and analysis of implanted medical devices and associated specimens that are removed from patients during revision surgery, at postmortem, or as part of animal studies. This practice can also be used for analysis of specimens and lubrication fluids from *in vitro* wear tests and joint simulators. The aim is to provide guidance in preventing damage to the associated specimens which could obscure the investigational results, and in gathering data at the proper time and circumstance to validate the study.

1.2 This practice offers guidelines for the analysis of retrieved implants to limit damage to them, and to allow comparisons between investigational results from different studies. The protocols are divided into three stages, where Stage I is the minimum non-destructive analysis, Stage II is more complete non-destructive analysis, and Stage III is destructive analysis. Standard protocols for the examination and collection of data are provided for specific types of materials in relation to their typical applications. For particular investigational programs, additional, more specific, protocols may be required. If special analytical techniques are employed, the appropriate handling procedures must be specified.

1.3 This practice recommendation should be applied in accordance with national regulations or legal requirements regarding the handling and analysis of retrieved implants and excised tissues, especially with regard to handling devices which may become involved in litigation, as per Practice E860.

1.4 A significant portion of the information associated with a retrieved implant device is often at the device-tissue interface or in the tissues associated with the implant and related organ systems. Attention should be given to the handling of adjacent tissues, so as not to interfere with study of the particles in the adjacent tissue, a chemical analysis for the byproducts of degradation of the implant, or a study of the cellular response to the implant.

1.5 The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard.

1.6 *This standard may involve hazardous materials, operations, and equipment. As a precautionary measure, explanted devices should be sterilized or minimally disinfected by an appropriate means that does not adversely affect the implant or the associated tissue that may be subject to subsequent analysis. A detailed discussion of precautions to be used in handling of human tissues can be found in ISO 12891-1. This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.*

2. Referenced Documents

2.1 *ASTM Standards:*[2]

A262 Practices for Detecting Susceptibility to Intergranular Attack in Austenitic Stainless Steels

A751 Test Methods, Practices, and Terminology for Chemical Analysis of Steel Products

C20 Test Methods for Apparent Porosity, Water Absorption, Apparent Specific Gravity, and Bulk Density of Burned Refractory Brick and Shapes by Boiling Water

C158 Test Methods for Strength of Glass by Flexure (Determination of Modulus of Rupture)

C169 Test Methods for Chemical Analysis of Soda-Lime and Borosilicate Glass

C573 Methods for Chemical Analysis of Fireclay and High-Alumina Refractories (Withdrawn 1995)[3]

C623 Test Method for Young's Modulus, Shear Modulus,

[1] This practice is under the jurisdiction of ASTM Committee F04 on Medical and Surgical Materials and Devices and is the direct responsibility of Subcommittee F04.15 on Material Test Methods.

Current edition approved Sept. 1, 2013. Published October 2013. Originally approved 1978. Last previous edition approved in 2010 as F561 – 05a (2010). DOI: 10.1520/F0561-13

[2] For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

[3] The last approved version of this historical standard is referenced on www.astm.org.

and Poisson's Ratio for Glass and Glass-Ceramics by Resonance
C633 Test Method for Adhesion or Cohesion Strength of Thermal Spray Coatings
C674 Test Methods for Flexural Properties of Ceramic Whiteware Materials
C730 Test Method for Knoop Indentation Hardness of Glass
C1069 Test Method for Specific Surface Area of Alumina or Quartz by Nitrogen Adsorption
C1161 Test Method for Flexural Strength of Advanced Ceramics at Ambient Temperature
C1198 Test Method for Dynamic Young's Modulus, Shear Modulus, and Poisson's Ratio for Advanced Ceramics by Sonic Resonance
C1322 Practice for Fractography and Characterization of Fracture Origins in Advanced Ceramics
C1326 Test Method for Knoop Indentation Hardness of Advanced Ceramics
C1327 Test Method for Vickers Indentation Hardness of Advanced Ceramics
D256 Test Methods for Determining the Izod Pendulum Impact Resistance of Plastics
D412 Test Methods for Vulcanized Rubber and Thermoplastic Elastomers—Tension
D570 Test Method for Water Absorption of Plastics
D621 Test Methods for Deformation of Plastics Under Load (Withdrawn 1994)[3]
D624 Test Method for Tear Strength of Conventional Vulcanized Rubber and Thermoplastic Elastomers
D638 Test Method for Tensile Properties of Plastics
D671 Test Method for Flexural Fatigue of Plastics by Constant-Amplitude-of-Force (Withdrawn 2002)[3]
D695 Test Method for Compressive Properties of Rigid Plastics
D732 Test Method for Shear Strength of Plastics by Punch Tool
D747 Test Method for Apparent Bending Modulus of Plastics by Means of a Cantilever Beam
D785 Test Method for Rockwell Hardness of Plastics and Electrical Insulating Materials
D790 Test Methods for Flexural Properties of Unreinforced and Reinforced Plastics and Electrical Insulating Materials
D792 Test Methods for Density and Specific Gravity (Relative Density) of Plastics by Displacement
D1004 Test Method for Tear Resistance (Graves Tear) of Plastic Film and Sheeting
D1042 Test Method for Linear Dimensional Changes of Plastics Caused by Exposure to Heat and Moisture
D1238 Test Method for Melt Flow Rates of Thermoplastics by Extrusion Plastometer
D1239 Test Method for Resistance of Plastic Films to Extraction by Chemicals
D1242 Test Methods for Resistance of Plastic Materials to Abrasion (Withdrawn 2003)[3]
D1505 Test Method for Density of Plastics by the Density-Gradient Technique
D1621 Test Method for Compressive Properties of Rigid Cellular Plastics
D1622 Test Method for Apparent Density of Rigid Cellular Plastics
D1623 Test Method for Tensile and Tensile Adhesion Properties of Rigid Cellular Plastics
D1708 Test Method for Tensile Properties of Plastics by Use of Microtensile Specimens
D2240 Test Method for Rubber Property—Durometer Hardness
D2842 Test Method for Water Absorption of Rigid Cellular Plastics
D2857 Practice for Dilute Solution Viscosity of Polymers
D2873 Test Method for Interior Porosity of Poly(Vinyl Chloride) (PVC) Resins by Mercury Intrusion Porosimetry (Withdrawn 2003)[3]
D2990 Test Methods for Tensile, Compressive, and Flexural Creep and Creep-Rupture of Plastics
D3016 Practice for Use of Liquid Exclusion Chromatography Terms and Relationships
D3417 Test Method for Enthalpies of Fusion and Crystallization of Polymers by Differential Scanning Calorimetry (DSC) (Withdrawn 2004)[3]
D3418 Test Method for Transition Temperatures and Enthalpies of Fusion and Crystallization of Polymers by Differential Scanning Calorimetry
D3835 Test Method for Determination of Properties of Polymeric Materials by Means of a Capillary Rheometer
D3919 Practice for Measuring Trace Elements in Water by Graphite Furnace Atomic Absorption Spectrophotometry
D4000 Classification System for Specifying Plastic Materials
D4001 Test Method for Determination of Weight-Average Molecular Weight of Polymers By Light Scattering
D4065 Practice for Plastics: Dynamic Mechanical Properties: Determination and Report of Procedures
D4754 Test Method for Two-Sided Liquid Extraction of Plastic Materials Using FDA Migration Cell
D5152 Practice for Water Extraction of Residual Solids from Degraded Plastics for Toxicity Testing (Withdrawn 1998)[3]
D5227 Test Method for Measurement of Hexane Extractable Content of Polyolefins
D5296 Test Method for Molecular Weight Averages and Molecular Weight Distribution of Polystyrene by High Performance Size-Exclusion Chromatography
E3 Guide for Preparation of Metallographic Specimens
E7 Terminology Relating to Metallography
E8 Test Methods for Tension Testing of Metallic Materials
E10 Test Method for Brinell Hardness of Metallic Materials
E18 Test Methods for Rockwell Hardness of Metallic Materials
E45 Test Methods for Determining the Inclusion Content of Steel
E92 Test Method for Vickers Hardness of Metallic Materials (Withdrawn 2010)[3]
E112 Test Methods for Determining Average Grain Size
E120 Test Methods for Chemical Analysis of Titanium and Titanium Alloys (Withdrawn 2003)[3]

E135 Terminology Relating to Analytical Chemistry for Metals, Ores, and Related Materials
E168 Practices for General Techniques of Infrared Quantitative Analysis (Withdrawn 2015)[3]
E204 Practices for Identification of Material by Infrared Absorption Spectroscopy, Using the ASTM Coded Band and Chemical Classification Index (Withdrawn 2014)[3]
E290 Test Methods for Bend Testing of Material for Ductility
E353 Test Methods for Chemical Analysis of Stainless, Heat-Resisting, Maraging, and Other Similar Chromium-Nickel-Iron Alloys
E354 Test Methods for Chemical Analysis of High-Temperature, Electrical, Magnetic, and Other Similar Iron, Nickel, and Cobalt Alloys
E386 Practice for Data Presentation Relating to High-Resolution Nuclear Magnetic Resonance (NMR) Spectroscopy
E407 Practice for Microetching Metals and Alloys
E562 Test Method for Determining Volume Fraction by Systematic Manual Point Count
E663 Practice for Flame Atomic Absorption Analysis (Withdrawn 1997)[3]
E860 Practice for Examining And Preparing Items That Are Or May Become Involved In Criminal or Civil Litigation
E883 Guide for Reflected–Light Photomicrography
E986 Practice for Scanning Electron Microscope Beam Size Characterization
E1188 Practice for Collection and Preservation of Information and Physical Items by a Technical Investigator
E1479 Practice for Describing and Specifying Inductively-Coupled Plasma Atomic Emission Spectrometers
F316 Test Methods for Pore Size Characteristics of Membrane Filters by Bubble Point and Mean Flow Pore Test
F619 Practice for Extraction of Medical Plastics
F981 Practice for Assessment of Compatibility of Biomaterials for Surgical Implants with Respect to Effect of Materials on Muscle and Bone
F1044 Test Method for Shear Testing of Calcium Phosphate Coatings and Metallic Coatings
F1147 Test Method for Tension Testing of Calcium Phosphate and Metallic Coatings
F1854 Test Method for Stereological Evaluation of Porous Coatings on Medical Implants
F1877 Practice for Characterization of Particles
F2102 Guide for Evaluating the Extent of Oxidation in Polyethylene Fabricated Forms Intended for Surgical Implants
F2182 Test Method for Measurement of Radio Frequency Induced Heating On or Near Passive Implants During Magnetic Resonance Imaging
F2214 Test Method for ***In Situ*** Determination of Network Parameters of Crosslinked Ultra High Molecular Weight Polyethylene (UHMWPE)
F2995 Guide for Shipping Possibly Infectious Materials, Tissues, and Fluids

2.2 *Other Document:*[4]
ISO 12891-1, Retrieval and Analysis of Implantable Medical Devices, Part 1: Standard Practice for Retrieval and Handling

3. Terminology

3.1 *Definition of Terms Specific to Issues of Microbial Contamination:*

3.1.1 *antiseptic*—a germicide that is used on skin or living tissue for the purposes of inhibiting or destroying microorganisms.

3.1.2 *decontamination*—a process or treatment that renders a medical device, instrument, or environmental surface safe to handle. Ranges from sterilization to cleaning with soap and water.

3.1.3 *disinfectant*—a germicide that is used solely for destroying microorganisms on inanimate objects.

3.1.4 *disinfection*—generally less lethal than sterilization. It eliminates virtually all recognized pathogenic microorganisms but not necessarily all microbial forms (for example, bacterial endospores) on inanimate objects. It does not ensure overkill.

3.1.5 *sterilization*—use of a physical or chemical procedure to destroy all microbial life; including large numbers of highly resistant bacterial endospores.

4. Summary of Practice

4.1 This practice provides recommendations for collection of clinical data, analysis of adjacent tissues, and the material characterizations to be performed when an implant is retrieved as part of a clinical or an animal study. It also provides for analysis of specimens and lubrication fluids from *in vitro* wear tests.

4.2 The clinical data to be recorded include a case history review, roentgenogram reviews, tissue culture, and observations of the implant site.

4.3 Protocols are provided for the handling of the implant tissue interface, and adjacent tissues and fluids for subsequent analysis. These protocols are intended to facilitate *(a)* histologic and immunohistochemical examination of the tissues, *(b)* chemical analysis of the tissues for identification and quantification of implant corrosion or degradation products, and *(c)* digestion of tissues and fluids for subsequent harvesting and analysis of particulate debris.

4.4 The material characterizations include observation and description of the retrieved device and adjacent tissues, determination of chemical composition, macroscopic and microscopic examinations and mechanical property determinations. The guidelines are separated in three stages. Stage I is considered to comprise an essential minimum analysis for routine examination of all types of materials. Stage II is nondestructive but provides more detail and is intended for special studies of devices with or without impaired function, made of all types of materials. Stage III includes destructive

[4] Available from American National Standards Institute (ANSI), 25 W. 43rd St., 4th Floor, New York, NY 10036, http://www.ansi.org.

methods for and material-specific protocols for detailed failure, microstructural, and chemical analysis as well as determination of physical and mechanical properties.

5. Significance and Use

5.1 The investigation of retrieved implantable medical devices and adjacent tissues can be of value in the assessment of clinical complications associated with the use of a specific prosthetic device design; can expand the knowledge of clinical implant performance and interactions between implants and the body; provide information on implant performance and safety; and thus further the development of biocompatible implant materials and devices with improved performance. Comparison of wear patterns and wear particle morphology observed with retrievals and those observed with *in vitro* joint simulator tests can provide valuable insight into the validity of the *in vitro* simulation.

5.2 A significant portion of the information associated with a retrieved implant is obtained with detailed studies of the device-tissue interface. Appropriate methods are provided to facilitate a study of the particles in the tissues, and chemical analysis for the byproducts of degradation of the implant, and histologic evaluation of the cellular response to the implant.

5.3 For the analysis to be accurate, it is essential that the device and associated tissues be removed without alteration of their form and structure. It is also essential that the tissues be handled in such a way as to avoid microbial contamination of the work place or the investigator. Standard protocols for the examination and collection of data are provided for retrieval and handling of implantable medical devices, as well as for specific types of materials in relation to their typical applications. For particular investigational programs, additional, more specific, protocols may be required. If special analytical techniques are employed, the appropriate procedures must be specified.

5.4 In order to interpret the analysis of materials and tissues, it is also essential to capture a minimum data set regarding the clinical findings and laboratory studies documenting device performance and reasons for removal.

5.5 Any destructive analysis of implants must be done so as to not destroy any features that may become the subject of litigation, as per Practice E860. This standard recommendation should be applied in accordance with state or national regulations or legal requirements regarding the handling and analysis of retrieved implants and tissues.

6. Interferences

6.1 Some critical features of the retrieved implant, tissue and the interface can only be accurately described by observation at the time of removal, and prior to sterilization or disinfection. Such observation must be made using appropriate aseptic precautions.

6.2 Due to the destructive nature of some of the analysis protocols provided in this practice, their use precludes any other type of analysis. It is therefore essential that handling of the device and tissues be done in concert with the requirements of all of the analyses to be performed, including analyses that may be done in the future. When harvesting tissues for subsequent chemical analysis, it is important to use tools that do not contain the materials or elements of interest in the tissues.

7. Hazards

7.1 The handling of retrieved implants and tissues may involve handling of infectious material.

7.2 It is suggested that individuals handling the devices be vaccinated against Hepatitis B. As a precautionary measure, removed implants should be sterilized by an appropriate means that does not adversely affect the implant.

7.3 There are situations where tissues or implants can not be sterilized or disinfected prior to analysis, for example, requirements of specialized protocols in which sterilization will adversely effect tissue or material properties. In such cases, extreme care should be taken to use aseptic technique and disinfection. Where institutional guidelines for the handling of septic material do not exist, details for handling and sterilizing retrievals, and laboratory practice recommendations can be found in ISO 12891-1.

8. Clinical Information Gathered at the Time of Implant Explantation

8.1 The extent of clinical information to be obtained will depend in part on the type of implant and reasons for removal. Similarly, the amount of information provided about the implant site will depend on the circumstances regarding the removal. A detailed listing and format for documentation of the clinical information associated with removal are provided in Appendix X1. Standard patient evaluation scoring schemes such as those developed by clinical societies may also be utilized.

8.2 As a minimum, the clinical information for device tracking should include the following information:

8.2.1 Date of implantation, and date of explantation.

8.2.2 Identification of hospitals, or physicians' offices, where device implantation and removal was performed.

8.2.3 Confidential, unique, patient ID Code to link to hospitals implantation and removal records.

8.2.4 Device identification (manufacturer's name and device catalogue number).

8.2.5 Device lot and serial number.

8.2.6 Indication for use and reason for explantation (clinical diagnosis).

8.3 For purposes of implant retrieval studies, the following information is considered essential:

8.3.1 Patient or animal age and sex.

8.3.2 A generic statement as to level of patient activity relative to the device.

8.3.3 A statement as to any gross evidence of inflammation, implant site infection, or tissue damage such as osteolysis.

8.3.4 Orientation of the implant relative to the patient. It is suggested that the proximal end of the device be identified with a nondestructive marking scheme.

8.4 More detailed clinical information should be gathered, where feasible, as indicated in Appendix X1. Obtaining an *in situ*, intraoperative photograph of the implant is highly desirable.

8.5 To facilitate subsequent analysis, it is recommended that the device be removed with the tissue interface intact. However, interface preservation should not jeopardize the practice of medicine and patient safety.

8.5.1 In cases of animal studies of tissue responses to implants, the implant should be removed with at least a 4 mm thick layer of adjacent tissue, as per Practice F981.

9. Analysis of the Tissues and the Tissue-Implant Interface

9.1 *Macroscopic Examination of Tissue:*

9.1.1 Record a gross pathologic description of the tissue immediately adjacent to the implant, as to consistency and color, as seen by the naked eye, or with a hand lens or dissecting microscope. Record any differences between the implant-tissue interface and the tissues not in direct contact with the implant. Describe the specimen size either by dimensions or weight.

9.1.2 Since the color of tissue is altered by sterilization and fixation methods, it is recommended that gross observations be made prior to sterilization. Such observations should be made utilizing aseptic techniques.

9.1.3 Where appropriate and feasible, obtain photographic documentation of the explant and adjacent tissue, as well as a photographic record of subsequent dissections.

9.2 *Histopathological Analysis of Tissue:*

9.2.1 Process the excised tissue using standard laboratory procedures for the histological dehydration, embedding and sectioning. These procedures may be for paraffin embedding, methacrylate embedding or other special procedures. Routine staining with hematoxylin and eosin (H & E), or toluidine blue are recommended for light microscopy of soft tissues and bone. Special stains, for example, von Kassa, Masson, Movat pentachrome, may be utilized as indicated and should be fully described.

9.2.2 Provide a detailed histopathologic description of the tissue-implant interface as well as all adjacent tissue specimens, for example, extracellular matrix, necrotic changes, thickness of fibrous capsule, cell types, particulates, hyperplasia, dysplasia, type of inflammatory reaction.

9.2.3 If the implant material is porous, then tissue analysis must include evaluation of the reaction within the pores as well as in the adjacent tissues. This should include the degree and nature of tissue ingrowth, and biological fixation.

9.2.4 For detailed studies of tissue reactions, the use of a quantitative scoring scheme, such as that in Practice F981 is recommended.

9.2.5 Since some polymeric materials, for example, PMMA bone cement, are altered or dissolved by the solutions used for routine histology, special techniques may be indicated, or special note made of voids formerly occupied by the material.

9.3 *Immunohistochemical and Other Special Histopathology Protocols:*

9.3.1 These procedures can be used for identifying specific cell types and extracellular matrix tissue responses to implantable materials and prosthetic devices. This field is constantly changing, and therefore only one such approach is provided as an example.

9.3.1.1 Typical markers chosen are for the presence of immunoglobulins on lymphocytes to indicate B cells or on monocytes/macrophages to indicate activation, the presence of CD2 markers to indicate immature T cells, the presence of CD3 markers to indicate mature T cells, and markers to indicate activated macrophages.

9.3.1.2 The protocols consist of a series of steps or reactions which have been developed to amplify the reactions, and to be cost effective. First, an antibody specific for the CD marker is used (typically mouse anti-human). Then, a biotinilated antibody to the first antibody is applied (typically goat anti-mouse); biotin serves as a marker in this amplification phase of the reactions. Strept-avidine peroxidase is then added to bind to the biotin and immobilize the peroxidase. Finally, a substrate is added which will react with the peroxidase, change color and precipitate. Diaminobenzidine (DAB) is often used, although several substrates are available for different kits or automatic systems. The end result is the peroxidase oxidation of DAB to give a yellow-brown precipitate at the site of the reaction. The sections can be stained with hematoxylin to enhance the visibility of cells.

9.3.1.3 An example of a method to be used is briefly summarized below and is based on standard techniques. Although it was originally described for use on frozen tissues, the use of embedded tissues allows for examination of the same tissue blocks used for routine pathology. This is only one of many approaches.

9.3.2 *Reagents:*

9.3.2.1 The reagents used come from a variety of companies including DAKO, Becton Dickinson, Kirkegaard & Perry, and Oncogene.

9.3.2.2 Antibody for specific markers, for example, CD2, CD3.

9.3.2.3 Biotinilated goat anti-mouse or anti-rabbit IgG.

9.3.2.4 Strept-avidine peroxidase.

9.3.2.5 Diaminobenzidine (DAB), or other suitable substrate.

9.3.3 Sections are deparaffinated in xylene for 5 min twice, and then rehydrated with absolute ethanol for 3 min, 95 % ethanol for 3 min, and then in 70 % ethanol for 3 min.

9.3.4 The sections are then placed in a methanol-hydrogen peroxide solution for 30 min to diminish the background level of peroxidase in the tissue. The sections are rinsed in water, next placed in buffered saline, and then the slide around the section is dried.

9.3.5 The slide is then placed in a humidity chamber, covered with buffer, and the first antibody is added. This will be the antibody specific for the marker (for example, CD2) and will be either of mouse or rabbit origin. This is incubated overnight, then rinsed with buffer, drained, and the slide around the tissue dried.

9.3.6 The second antibody, which is biotinolated, is added. This is usually goat anti-mouse or anti-rabbit IgG. This is

incubated for 30 min, rinsed, the slide dried, and then strept-avidin peroxidase is added.

9.3.7 The strept-avidin peroxidase is incubated for 30 min, rinsed, and then a substrate such as DAB is added. The development of the color is watched under the microscope, the action stopped with water, then the slides are dipped into osmium tetroxide for final fixation. The slides may be counterstained with hematoxylin for visualization of all cells. The slides are processed for mounting with eukitt and can be evaluated for presence of label.

9.3.8 This method can be used to detect the production of cytokines in the cells in the tissues. However, caution should be used in the interpretation of findings, since these are soluble mediators and rapidly leave the site of origin.

9.4 *Chemical Analysis of Tissues By Flame Atomic Absorption Spectroscopy (AAS), Graphite Furnace Atomic Absorption Spectroscopy (GFAAS), by Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) or Mass Spectroscopy (ICPMS):*

9.4.1 *Reagents and Materials:*

9.4.1.1 Standard AAS grade solutions (MCB reagents, Fisher, and VWR) are used to make calibration curves. Calibration solutions should be prepared according to Practice D3919, using the same matrix solution as the test specimen. Solutions of low concentration should be made fresh daily. The sensitivity and possible interferences depend on the particular element.

9.4.1.2 Any fixing agents, chemicals and solvents must be of analytic purity. The use of 70 % ethanol is recommended as a transport and storage solution. The use of double distilled, deionized water is necessary.

9.4.1.3 Handling of tissues for subsequent chemical analysis requires special precautions to be taken to insure that the specimens are not contaminated with the elements to be analyzed. Surgical knives or instruments used for tissue excision shall be free of any contamination or loose particulates. The use of ceramic or glass knives is recommended for preparation of specimens associated with metallic implants. Glass knives are not recommended for subsequent silicone analysis.

9.4.1.4 Tissues should be transferred to plastic or glass containers of high quality which have been thoroughly acid cleaned or unused from a lot tested to be free of contamination. Acid cleaning which may etch the glass surfaces is not recommended for subsequent silicone analysis. Tissue transfer should be done in a dust free environment.

9.4.2 Test solutions should be analyzed in triplicate, either as is or after dilution with 1 % nitric acid to a concentration which falls within the standards, and the results averaged. Concentrations are determined in µ/l (ppb), or µ/g of tissue (ppm). Results from solutions of known volumes from *in vitro* studies can be converted to total micrograms in solution.

9.4.2.1 The concentration of metallic species in tissue may vary according to the location of the specimen relative to the implant. It is therefore important to carefully record the location of the specimen.

9.4.3 These methods of analysis require chemical digestion of the tissue samples prior to analysis, and therefore the samples can not be used for any other analysis. The ability to digest tissue is influenced by the method of tissue fixation. It is recommended that tissues be fixed in analytical grade 70 % ethanol in analytical grade water. The methods of digestion depend on the type of tissue to be analyzed.

9.4.3.1 *Blood* samples drawn from patients or animals should be done using polypropylene syringes. The blood can be allowed to clot at room temperature and centrifuged at 1850 g for 30 min to separate serum and clot fractions. Blood may also be drawn in heparinized vacutainer tubes. The blood may be allowed to settle so as to isolate red and white cells, or be centrifuged at 400 g and the plasma supernatant drawn off. Plasma is diluted at least 2× in 1 % nitric acid.

9.4.3.2 *Cells,* either red blood cells or cells from cell culture experiments may receive special treatment, such as separation of cell contents and cell membranes. The cells are washed and centrifuged 3 times with physiologic saline to remove trapped serum or growth media. The cell pellet is then lysed with 1 % Triton X100 to release intracellular contents, centrifuged and the supernatant harvested. This solution is pipetted off, diluted 2× in 1 % nitric acid, and referred to as "cell contents." The pellet of cell membranes is then washed and centrifuged 3 times in saline to remove the Triton and remaining contents. The membranes are then digested in 50 % nitric acid, diluted in 0.5 % nitric acid for analysis as "cell membranes."

9.4.3.3 *Tissue* from implant sites or joint capsules should be weighed and placed in plastic bags.

(1) For the mechanical tissue digestion appropriate bags for the machine should be used. These may then be frozen until use. For preparation, 5 mL of 50 % nitric acid will be added to each bag. The tissue is then homogenized in a mechanical blender.

(2) Alternatively, tissue should be placed in analytical grade 70 % ethanol in analytical grade water in the proper container that will not allow cross-contamination.

(3) The tissue sample may be dried (15 min at 90°C) prior to digestion, to determine the dry weight. Acid digestion of the dry sample can then be accomplished with nitric acid. The dried tissue sample (~100 mg) should be mixed with 5 mL of low trace nitric acid (minimum 70 % HNO_3) and heated for approximately 2 h (or more if needed) at 90°C. Alternatively, the same solution can be placed in a microwave digestion bomb (that is, Parr Microwave Digestion Bombs Model No. 4781 23 mL or 4782 45 mL),[5] which is a chemically inert vessel designed for high heat where venting may be required. These vessels can be placed in a household microwave for more rapid sample dissolution (that is, 2 min at medium power). There are also commercial microwave digestions systems available that operate at higher pressures and temperatures which also have vessels and carrousels as part of the system. (See Note 1.)

(4) The samples are then pipetted into the GFAAS or ICPMS for analysis. Dilution as necessary is done with nitric

[5] The sole source of supply of the apparatus known to the committee at this time is Parr Instrument Co., 211 Fifty Third St., Moline, IL 61265–9984. If you are aware of alternative suppliers, please provide this information to ASTM International Headquarters. Your comments will receive careful consideration at a meeting of the responsible technical committee,[1] which you may attend.

acid. Following the digestion procedure, the solution may need to be diluted to a pre-determined amount (that is, 5:1) using de-ionized water. Dilutions may be necessary if the analytical equipment cannot operate with samples containing high acid concentration.

NOTE 1—Microwave digestion is a good alternative to traditional acid digestion, since microwave dissolution is faster, safer, and simpler, and provides more controlled reproducible conditions than conventional methods. Depending on the power ratings of the microwave, an appropriate level will need to be chosen in conjunction with the adequate time to achieve full digestion of the tissue.

9.4.3.4 *Bone* specimens can be subjected to a two-stage digestion procedure to separate them into two phases, mineralized and demineralized (or organic). The bones are placed in 0.5 N HCl for 48 hrs at 4°C to demineralize them. The rubbery demineralized samples are then placed in 50 % nitric acid to digest the organic portion. Both the HCl and HNO_3 samples are diluted as necessary and analyzed by GFAAS.

9.4.4 Chemical analysis by flame AAS should be done according to manufacturer's instructions, in accordance with E663 and E135.

9.4.5 Analysis with graphite furnace (GFAAS) should be done according to Practice D3919 using the manufacturer's specifications for analyte wavelength and slit width. Calibration standards should be made up in the same matrix as the test specimens.

9.4.5.1 A multi-cycle protocol is recommended to insure complete drying of the specimen prior to charring and atomization; additional steps may be required for post analysis clearing of the tubes.

9.4.5.2 The use of ultra pure argon for clearing is essential.

9.4.5.3 There are two types of graphite tubes used in the furnace: pyrolytic carbon coated graphite tubes, and tubes with L'vov platforms. The analysis for nickel, molybdenum, platinum, titanium, and vanadium are done from the wall of the tubes while cobalt, chromium, and aluminum are done on platforms.

9.4.6 Chemical analysis by ICP should be done in accordance with Practice E1479.

10. Analysis of Tissues and Fluids for Particulate Debris

10.1 Analysis for particulates can be done histopathologically as in 9.2, or by tissue or fluid digestion and particle separation. Tissues subjected to digestion become dedicated to this type of analysis.

10.1.1 Preparation of tissue for drying and digestion will depend on the state of the tissue. If the tissue is embedded in paraffin, the tissue block should be deparaffinized with xylene overnight at room temperature, and then washed with 100 % ethanol. If tissue is fixed in formalin, it should be dehydrated through a series of alcohols using standard histological protocol, and infiltrated with 100 % ethanol. If tissue is fresh, it should be frozen and free of embedding media.

10.1.2 The tissue will be weighed, or the fluid volume determined before digestion. If a wet weight is desired (only applicable for the fresh tissue), 0.3–0.5 g are typically weighed out. For a dry tissue weight, the tissue should be freeze dried, sliced with a ceramic knife, and 0.02–0.03 g weighed out with a microbalance. Weighed tissue should then be placed in acid washed polystyrene vials. Once weighed, the tissue is suitable for digestion.

10.1.3 Several digestion protocols are described in the literature. The choice of protocol depends in part on the type of particle of interest, for example, metal versus polymer, and on the type of tissue. Four methods of digestion are described in this recommended practice.

10.2 *Reagents for Digestion:*

10.2.1 *Ultrapure water*—Distilled H_2O filtered with 0.2 µm filter.

10.2.2 *Phosphate Buffer*—3.55 g Na_2HPO_4, 3.45 g NaH_2PO_4, 0.744 g EDTA, in 100 ml ultrapure water.

10.2.3 *Papain solution*—1 ml phosphate buffer, 100 µL pure papain, 3.26 mg N-acetylcysteine, 9 ml ultrapure water.

10.2.4 *Strong bases*—Tissues have been digested in solutions of sodium hydroxide ranging from 1–10 N NaOH (5–50 ml/g of tissue), or in potassium hydroxide 2 N KOH (10 ml/g of tissue), or 4 M KOH (2 ml/gram of tissue).

10.2.5 *Pronase*—2000 U/ml, 50 mM Tris, 75 mM NaCl.

10.2.5.1 Concentrated nitric acid (HNO_3) has been used when tissues are difficult to digest, especially after fixation and embedding.

10.3 *Procedure for Digestion in Papain:*

10.3.1 Place 1 gram of tissue sample in a clean 50 ml conical tube.

10.3.2 Add 5 ml of papain solution and vortex.

10.3.3 Incubate sample at 65°C for 24 h. If sample does not dissolve add an additional 100 µl papain and vortex.

10.3.4 Centrifuge in an ultracentrifuge for 1 h at 100 000 g. A variable gradient may be used comprising of 2.0 ml each of 5, 10, 20, and 50 % sucrose.

10.3.5 Wash particles in 10 cc of hot filtered water.

10.4 *Procedure for Digestion in Strong Base and Pronase:*

10.4.1 Rinse tissue samples in phosphate buffered saline, and place 1 g in a 15 ml glass tube.

10.4.2 Add 2 ml of 4 M KOH.

10.4.3 Incubate sample at 56°C for 48 h. Mix samples occasionally.

10.4.4 Centrifuge at 1000 g for 1 h. To facilitate sedimentation of debris, add 6 ml of 95 % ethanol to the 2 ml aliquots. Discard clear supernatant, and repeat digestion and centrifugation steps.

10.4.5 Wash debris in distilled water, mix and place in 37°C for 8 h. Mix with excess amounts of ethanol, and centrifuge at 1000 g, 1 h. Repeat washing procedure 3 times.

10.4.6 Digest the organic material with debris in pronase at 37°C for 24 h. During the first 5 min in pronase, ultrasonicate the debris to disaggregate the particles.

10.4.7 Wash the debris 3 times in distilled water.

10.4.8 Resuspend debris (0.5 ml debris/3 ml of ethanol) and add an equal volume of hexane and vortex.

10.4.9 Transfer the cream colored fraction containing the polymeric particles, which stabilized at the hexane-ethanol interface into a clean, sterile tube. Metal debris and bone particles will be in the sediment at the bottom.

10.4.10 Repeatedly add hexane or ethanol to the original tube and vortex. Harvest and pool the polyethylene particles.

10.4.11 To the pooled particles, add 2 ml of ethanol and evaporate any remaining hexane.

10.4.12 Add excess ethanol to the tube and centrifuge at 1000 g for 30 min.

10.4.13 Discard the supernatant and resuspend debris in KOH at 56°C for 8 h.

10.4.14 Add excess ethanol to the tube and centrifuge at 1000 g for 30 min.

10.4.15 Discard the clear supernatant and resuspend particles in 1 ml of distilled water, incubate for 8 h followed by centrifugation in ethanol. Repeat wash procedure three times.

10.4.16 Resuspend approximately 0.5 ml of debris in 2 ml of ethanol. Ultrasonicate for 5 min before transfer to a stub for SEM and particle analysis.

10.5 *Procedure for Digestion in Nitric Acid:*

10.5.1 With this protocol, the digest solution is never filtered, therefore, the submicron particles are not lost and can be evaluated (with SEM and Coulter), without complications that could arise from hemosiderin particles which remain with non-acid digestion protocols.

10.5.2 If paraffin embedded sections are deparaffinated in xylene or toluene, replace the solution with 2 changes of absolute ethanol.

10.5.3 Critical point dry the tissue specimen, slice it and place 0.02 to 0.03 g pieces in plastic tubes.

10.5.4 Add 1.0 ml of concentrated nitric acid.

10.5.5 Digest the tissue for 24 h at room temperature. Shake the tube and sonicate for 2 min. Continue digestion for an additional 24 h.

10.5.6 Centrifuge digestion solution at 9500 g for 5 min. Depending on the amount of debris, there will be a floating band of polymeric debris and a sedimented pellet of metallic debris. These can either be isolated separately, or together.

10.5.7 Separate the fluid from debris by aspirating the clear liquid between the floating band and the pellet.

10.5.8 Add 1 ml of concentrated nitric acid to the debris, sonicate for 2 min and centrifuge at 11 600 rpm for 5 min.

10.5.9 Aspirate the clear liquid and add 1 ml of acetone, sonicate 2 min, and centrifuge at 11 600 rpm for 20 min. Both metallic and polymeric debris will sediment in the acetone.

10.5.10 Aspirate the supernatant and resuspend the debris in 10 µl of dispersant (Coulter I B) and slowly add 1 ml of ultrapure water, with intermittent sonication.

10.6 *Procedure for Tissue or Dried Lubricant or Synovial Fluid Digestion in Sodium Hydroxide:*

10.6.1 For tissue only: mince 2.0–5.0 g of tissue and place in a glass container. To extract lipid, add 2:1 chloroform-:methanol solution and place on an orbital shaker overnight, or until the tissues sink to the bottom of the container. Rinse tissues with 3× filtered (0.2 µm) deionized H_2O.

10.6.2 For extracted tissues and dried (lyophilized) fluid, add 12 ml 5*N* NaOH and incubate at 65°C for 1 to 3 hours on a water bath-shaker.

10.6.3 Allow the digested solution to cool to room temperature, then ultrasonicate for 10 minutes.

10.6.4 Into two clean, particle-free polyallomer centrifuge tubes, place 7 ml each of the digested solution and top off with 5 ml of 5 % sucrose. Ultracentrifuge for 3 hours at 40 000 rpm, 5–15°C.

10.6.5 UHMWPE will rise to the top of each tube to form an opaque layer. Carefully pipette this band into another clean, particle-free vial. Other bands that sometimes appear can be collected separately or pooled with the UHMWPE.

10.6.6 To wash off the sucrose, add 3× filtered deionized H_2O to the collected band until the total volume is 21 ml. Ultrasonicate for 5 min, then heat sample for 1 h at 80°C.

10.6.7 Into three clean, particle-free polyallomer centrifuge tubes, place 7 ml each of the solution and top off with an isopropanol gradient consisting of 3 ml of 0.96 g/cm^3 isopropanol/deionized H_2O and 2 ml of 0.90 g/cm^3 solution. Ultracentrifuge for 1 h at 40 000 rpm, 20–25°C.

10.6.8 Collect the UHMWPE band at the 0.90 and 0.96 g/cm^3 interface into a clean, particle-free vial. Other bands that may appear can be collected separately.

10.7 *Procedure for Sodium Hydroxide Digestion of Protein Solutions from Wear Tests:*

10.7.1 Lubrication fluids harvested during wear or joint simulation tests may be stored frozen at −20°C until digestion.

10.7.2 Digest a minimum volume of 80 ml serum or protein containing solutions by adding KOH pellets to a concentration of 12M. Digest at 60°C for 48 h or until solution clears.

10.7.3 The digested fluids are cooled to 4°C. Lipids and proteins are removed by the addition of an equal volume of chloroform:methanol (2:1). The solutions are then incubated at room temperature for 24 h, and then centrifuged at 2000 g for 10 min at RT. The contaminating lipids and proteins form a layer at the interface of the two solutions. The top layer containing the polyethylene wear particles is then decanted by pipetting into a clean tube and the procedure repeated three times or until the supernatant clears completely, that is, all visible lipids and proteins removed.

10.7.4 Any remaining proteins are removed by precipitation with the addition of an equal volume of ice-cold ethanol. The solutions are incubated at 4°C for 24 h with stirring and then centrifuged at 10 000 g at 4°C for 30 min. The fluid is carefully decanted to a clean vessel to avoid disturbing the protein pellet.

NOTE 2—This protocol will work well for isolation of UHMWPE particles. The enzyme digestion protocol in 10.3 may be found to work better for isolation of metal or ceramic debris in joint simulator fluids.

10.8 *Procedure for Hydrochloric Acid Digestion of Serum Proteins for Harvesting UHMWPE Particles from Wear Test Solutions:*

10.8.1 Lubrication fluids harvested during wear or joint simulation tests may be stored frozen at −20°C until digestion.

10.8.2 Add 4 to 5 parts of 37 % hydrochloric acid to 1 part of the simulator serum solution. (Volumes of 40 to 50 mL of acid to 10 mL of serum are suggested).

10.8.3 Heat the solution to 50 to 60°C for 45 min to 1 h while stirring with a magnetic stir bar at 350 rpm.

10.8.3.1 Digestion of serum protein is generally indicated by fluid becoming clear.

10.8.3.2 The time and temperature for full digestion may depend on the serum type and protein concentration.

10.8.4 Extract 1 mL of solution and add to 100 mL of methanol.

10.8.5 Vacuum filter the solution through a 47 mm diameter polycarbonate filter with a maximum pore size of 0.2 µm.

10.8.6 It has been found that UHMWPE wear particles can be significantly smaller than 0.2 mm in size. Therefore it is recommended that the filter pore size be 0.05 mm or smaller, depending on the application.

11. Analysis of Tissues and Fluids for Particulate Debris using Protocols that Directly Deposit Particles onto the TEM and/or SEM Grid or Wafer

The protocols of this section are adapted from those described in detail and validated in the pair of manuscripts: Billi *et al.* CORR, 2011, Part 1 and Part 2.

11.1 *Procedure for Digestion of Serum Proteins for Harvesting UHMWPE Particles from Wear Test Solutions*

11.1.1 Deionized water is ultrapurified (UP-dH2O) (18.2 MΩ; total organic content, < 5 ppb; pyrogens < 0.001 EU/mL).

11.1.2 All solutions used including UP-dH2O should be filtered through 0.02-µm[6] before use or lyophilization.

Digestion

11.1.3 Lubrication fluids harvested during wear or joint simulation tests shall be stored frozen at –20°C or lower until digestion.

11.1.4 Add 3 mL serum wear lubricant (a volume chosen to provide optimum digestion) to a 50-mL tube containing previously lyophilized 8 mol/L urea, 0.1 mol/L 2-[4-(2-hydroxyethyl)piperazin-1- yl]ethanesulfonic acid (HEPES) buffer (pH 7.5, optimal pH for proteinase activity), and 0.04 % NaN_3 (volume before lyophilization: 6 mL). If the lubricant samples contain ethylenediaminetetraacetic acid (EDTA), add 400 µL 0.5 mol/L $CaCl_2$ to overtitrate the EDTA and improve the activity of proteinase K (pK), which is stabilized in urea in the presence of Ca^{2+} (12–13 mmol/L).

11.1.5 Add 167 µL proteinase K (20 mg/mL) and incubate at 37°C for 18 hours with gyratory mixing (250 rpm).

11.1.6 Remove the sample from the gyrator and sonicate it with a probe four times for 30 seconds, separated by 1 min on ice.

11.1.7 Add 167 µL proteinase K and continue gyratory incubation at 37°C for another 24 hours.

11.1.8 Sonicate the sample again as in 11.1.6, and then add 167 µL proteinase K with gyratory mixing (250 rpm) for another 5 hours.

11.1.9 Repeat the sonication as in 11.1.6, and then add 750 µL 200 mmol/L EDTA (60 mmol/L final concentration) and 850 µL 0.5 mol/L tris(2-carboxyethyl)phosphine (TCEP), 0.1 mol/L HEPES, pH 7.0, 0.04 % NaN3 (final TCEP concentration, 20 mmol/L) to terminate the digestion during 3 additional hours of incubation.

11.1.10 Hold samples at 4°C for 18 hours before purification.

Purification and Display of the Particles

11.1.11 Step 1: Sonicate the sample digest as above and load 7 mL into the bottom of a 14-mL polyallomer centrifuge tube. Overlayer the sample, first with 2 mL buffered 6 mol/L urea and then with 3 mL of a solution containing 20 % sodium lauroyl sarcosine (SLS), 4 mol/L urea, 20 mmol/L EDTA, 50 mmol/L HEPES at pH 7.5, and 0.04 % NaN_3. After centrifugation at 284 000g for 4 hours at 37°C, collect the polyethylene (PE) particles at the liquid-air interface by cutting the tube 1.0 mm below the interface and rinsing the tube/cutter with 1.2 mL SLS/urea solution.

11.1.12 Step 2: Build a continuous isopropyl alcohol (IPA) gradient in a SW40-equivalent tube by sequential layering of 2 mL 20 % IPA, 2 mL 25 % IPA, 1.5 mL 30 % IPA, 1 mL 35 % IPA, and 0.5 mL 100 % IPA and allowing the tube to stand undisturbed for 18 hours at 4°C. Heat the sample from Step 1 at 80°C for 20 minutes and then sonicate without a probe[7] four times for 1 minute with an ice water step of 1 minute between sonications. Next, layer 2 mL 2 % SLS beneath the IPA gradient using a 3-mL syringe attached to a Pasteur pipet, followed by 1.5 mL 20 % SLS in 3 mol/L urea, and finally by 2 mL of the sample from Step 1. Centrifuge the tube at 4446 g for 30 minutes and then at 284 000g for 4 hours, all at 25°C. Collect the PE particles by cutting the tube above and below the isopycnic layer of opaque PE and rinsing the tube/cutter with 1.2 mL 40 % IPA. Dilute the sample with 100 % IPA (2:5) to a volume of 7 mL and hold for 18 hours at 4°C.

11.1.13 Step 3: Sonicate the sample with a probe (any sonicator, cell disruptor, or ultrasonic homogenizer probe should be adequate for this purpose) four times for 30 seconds, separated by 1 minute on ice, and then layer above 50 % (3 mL) and 10 % (2 mL) IPA. Centrifuge the tube at 284 000g for 5 hours at 25°C. Collect the particles at the interface between 50 % and 10 % IPA and store at 4°C for 18 hours.

For morphometric analysis, the particles are deposited onto a 5 × 5 mm silicon wafer that has been coated with a monolayer of marine mussel glue according to the following procedure:

11.1.14 First, clean the wafer by sonication in acetone: IPA (1:1) and then coat it with marine mussel glue.[8]

11.1.15 Add 10 µL marine mussel glue to a microfuge tube, followed by 200 µL 0.2 mol/L HEPES (pH 9.2), 0.15 mol/L NaCl, 0.04 % NaN_3.

11.1.16 Immediately after brief mixing by pipetting, uniformly spread 20 µL of this solution over the silicon wafer and incubate it for 30 minutes at 25°C in a Petri dish to prevent drying.

11.1.17 Remove excess glue by washing with 50 mmol/L HEPES (pH 7.5), 0.15 mol/L NaCl, 0.04 % NaN_3.

11.1.18 Use the wafer either immediately or store it up to 1 hour in 50 mmol/L HEPES (pH 7.5), 0.15 mol/L NaCl, 0.04 % NaN_3.

11.1.19 Sonicate the sample from Step 3 as in Step 3 and mix 125 µL of the sample with 750 µL filtered ultrapurified (UP-dH_2O), and add the mixture to a SW60 (or equivalent) 4.2-mL polyallomer centrifuge tube for flotation of the particles onto an inverted, coated silicon wafer that is positioned at the top of the tube with a custom-made polycarbonate holder (Fig. 1).

[6] Anodisc filters (Whatman International Ltd, Maidstone, UK) has been found satisfactory for this purpose

[7] UIS250v with Vial Tweeter; Hielscher or equivalent.

[8] Cell-Tak BD Biosciences, San Jose, CA have been found satisfactory for this purpose.

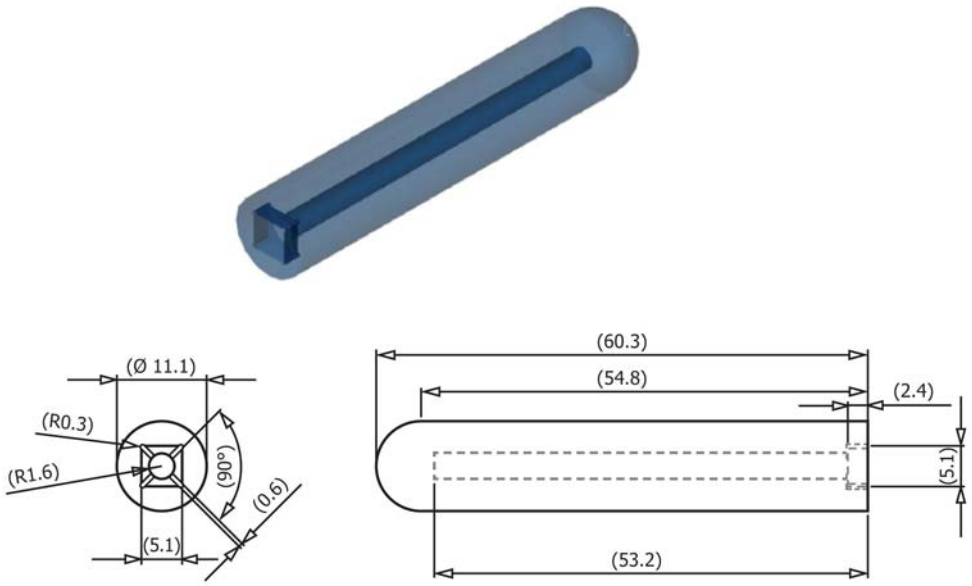

FIG. 1 Polycarbonate Holder for Silicon Wafer (Dimensions in Millimetres)

11.1.20 After centrifugation at 84 000 g for 4 hours, remove the wafer, gently wash it with UP-dH2O water, and dry it in a laminar flow hood to prevent contamination. The volume of the sample might need to be adjusted so that the particles collected on the wafer are well separated, with minimal particle clumping and overlaying.

11.1.21 The particles are verified as PE by the presence on the Fourier transform infrared spectrum of a carbonyl peak located between 1689 and 1756 cm^{-1}.

11.1.22 Given the area analyzed in the images and the cross-sectional area of the centrifuge tube, the approximate total number of particles in the tube can be calculated. Then, taking into account the dilutions, the approximate total number of particles in the original sample can be calculated. Dividing this by the number of cycles experienced by the implant to gives the approximate number of particles generated per cycle.

Rationale

Digestion of lubricant proteins with proteinase K in the presence of urea and calcium leads to more complete proteolytic digestion, and thus, denaturation of proteins due to urea-dependent cleavage of hydrogen bonds without the need for detergents. Inclusion of calcium during digestion partially protects proteinase K from auto-digestion in urea. After digestion, calcium is chelated with excess EDTA to reverse any divalent cation-dependent peptide linkages, and disulfide bonds are broken with TCEP; both steps lead to the smallest possible peptide digestion products. The 37°C temperature assures maximum activity for proteinase K under these conditions.

Purification of the particles is obtained using a three-step ultracentrifugation process. Step 1 utilizes stable reagent layers during centrifugation to minimize handling. Buoyant PE particles move upward in the tube, leaving peptides behind as they enter into the next peptide-free denaturing urea layer. The particles are ultimately deposited in a layer of concentrated detergent (SLS)/urea to solubilize lipids and disperse particles without aggregation. The stable separation of peptide and detergent, and the continuous washing of particles as they pass out of and into the reagent layers, avoids the formation of aggregates of peptides, particles and detergent, which can be difficult to break up.

Step 2 is preceded by heating the particles in detergent/urea to maximize the capacity of SLS to remove any remaining material adsorbed to the particles. In this centrifugation step, particles are floated out of 20 % SLS/urea, through a washing layer of SLS/urea, and through a layer of 2 % SLS. The particles then enter a continuous IPA gradient that serves to strip SLS from the particles and defines the buoyant density of the particles indicated by an opaque band.

Step 3 concentrates particles at the sharp 10 %:50 % IPA interface and further separates the particles from residual detergent. These purified particles are ready for characterization and use in other experimental procedures.

11.2 *Procedure for Digestion of Serum Proteins for Harvesting Ceramic and Metal Particles from Wear Test Solutions*

11.2.1 Deionized water is ultrapurified (UP-dH2O) (18.2 MΩ; total organic content, < 5 ppb; pyrogens < 0.001 EU/mL).

11.2.2 All solutions used including UP-dH2O should be filtered through 0.02-µm[9] before use or lyophilization.

Digestion

11.2.3 Lubrication fluids harvested during wear or joint simulation tests shall be stored frozen at –20°C or lower until digestion.

11.2.4 To concentrate the sample, centrifuge 36 mL (that is, the maximum volume of a SW32 tube) of wear lubricant or particle standard in a siliconized[10] SW32 (or equivalent) polyallomer centrifuge tube for 3 hours at 164 000 g and 25°C.

11.2.5 After centrifugation, remove the supernatant from the centrifuge tube, leaving 4 mL supernatant and the pellet.

[9] Anodisc filters (Whatman International Ltd, Maidstone, UK have been found satisfactory for this purpose.

[10] SurfaSil Siliconizing Fluid; Thermo Fisher Scientific Inc, Waltham, MA have been found satisfactory for this purpose.

11.2.6 For protease digestion, first, lyophilize 6 mL 8 mol/L urea (0.1 mol/L HEPES, pH 7.5) in a particle-free environment.

11.2.7 Add lyophilized urea solution, calcium chloride (final concentration, 40 mmol/L), and proteinase K (final concentration, 0.5 µg/mL) to the particle-containing SW32 tube from the concentration step.

11.2.8 Place the SW32 tube into a 50-mL capped conical centrifuge tube and then into a gyrator at 37°C and 250 rpm for 24 hours with periodic additions of proteinase K (167 µL to a final concentration of 0.5 mg/mL, at 0 and 18 hours) and sonication before each addition. Always sonicate in the sealed 50-mL tubes without a probe[11] to prevent introduction of metal debris shed from a probe, and sample cross-contamination. Sonicate four times at 1-minute intervals.

11.2.9 At the end of digestion, overtitrate the calcium with EDTA (to 24 mmol/L), and then add TCEP to 58 mmol/L as a metal nonreactive reducing agent.

Purification and Display of the Particles

Purification and display can be performed over two different supports: silicon wafers used for scanning electron microscopy (SEM) analysis and transmission electron microscopy (TEM) grids for TEM analysis.

11.2.10 For silicon wafers, repeat the procedure described in 11.1.14 – 11.1.18.

11.2.11 For TEM grids, coat the TEM grids with marine mussel glue.[12]

11.2.12 Dilute 10 µL marine mussel glue and mix it in 200 µL 0.2 mol/L HEPES (pH 9.2), 0.15 mol/L NaCl, 0.04 % NaN_3.

11.2.13 Spot 100 µl onto a sheet of parafilm. Then, invert the functional side of the TEM grid onto the bubble of glue and incubate for 30 minutes before washing it by swirling it in a Petri dish filled with 50 mmol/L HEPES (pH 7.5), 0.15 mol/L NaCl, and 0.04 % NaN_3.

11.2.14 Hold the TEM grid in a separate dish containing the same solution until needed (up to 1 hour).

11.2.15 Add 700 µL 2.0 g/mL cesium trifluoroacetate (CsTFA) to siliconized polyallomer ultracentrifuge tubes (4 mL) followed by a custom-made solid plug (plug density 2.2 g/mL, plug volume 0.9 mL).

11.2.16 Transfer the silicon wafer (or TEM grid) to the top of the plug and allow the plug and wafer (or grid) to sink to the bottom. The plug serves as a flat support for the wafer (or grid) and is firmly seated in the tube by centrifugation at 25°C and 30 000g for 15 minutes, before other reagents are added.

11.2.17 Sonicate samples from the digestion step in sealed tubes four times in 1-minute intervals, each in rotation.

11.2.18 Layer 1200 µL 7 mol/L urea, 20 mmol/L EDTA, 50 mmol/L HEPES, 0.04 % NaN3 above the CsTFA layer in the polyallomer tubes, followed by 1200 µL digested, sonicated sample. The volume of the sample might need to be adjusted so that the particles collected on the wafer are well separated, with minimal particle clumping and overlaying.

11.2.19 Centrifuge the step gradient first at 37°C for 30 minutes at 3300 g and then at 37°C for 4 hours at 84 000 g.

11.2.20 Cut the centrifuge tube in the middle of the CsTFA layer to isolate and remove the layers containing digestion contaminants.

11.2.21 For silicon wafers, extract the wafer from the cut section and wash it using a continuous stream of UP-dH2O in a dialysis machine for one hour at the rate of 20 mL per hour to remove any cesium salts. Alternatively, gently swirl the wafer in a petri dish containing UP-dH2O for one minute. Then, dry in a covered Petri dish in a laminar flow hood to prevent contamination.

11.2.22 For TEM grids, extract the TEM grids using reverse forceps and swirl them in two sequential extra-large Petri dishes filled with filtered UP-H2O before drying them for 15 minutes in a laminar flow hood and storing them in a sealed Petri dish.

Rationale

The concentration step generates a composite particle/protein pellet, which minimizes direct particle-particle interactions and agglomeration. Since this centrifugation step occurs in a low-density solution (about 1 g/mL), minimal particles are left behind due to protein-particle interactions. The concentrated particles never leave the tube until after protease digestion, minimizing particle loss.

For the digestion hold the same consideration made in case of polyethylene particles (Rationale, 11.1).

The use of a vial tweeter instead of a probe sonicator enhances the purity of the digested sample by preventing any contamination from particles that are usually shed from the probe in small but significant numbers.

The use of a monolayer of marine mussel glue causes particles to adhere to the wafer (or grid) surface so that they are not lost during removal of gradient solutions and other handling.

Siliconization prevents adhesion of particles to the wall of the tube.

During the low-speed stage of the purification step, the dense particles move through the digest and then through the cleaning digest-free urea layer at a slow, minimally convective speed. The high-speed phase moves the pure particles with a density greater than 2 g/mL through the CsTFA layer onto the glue-coated wafer or TEM grid (11.2.19).

One simple and routine indication of complete protein digestion is the absence of any bands of isodense metal-protein complexes in and above the CsTFA layer.

11.3 *Procedure for Digestion of Serum Proteins for Harvesting Particles from Tissues*

Sample Handling

11.3.1 Handling of tissue samples should be performed according to Guide F2995.

Digestion Protocol

11.3.2 Add 500 mg of tissue (an amount that has been found to work well in this protocol) to 6 mL of 8M urea/0.1 M Hepes/0.04 % NaN_3 (lyophilized) and add up UP-dH_2O to a final volume of 5.5 mL.

11.3.3 Add pK to a final concentration of 1 mg/mL (that is, always double pK concentration compared to that used for

[11] UIS250v with Vial Tweeter for 0.8 cycles at 100 % power has been found satisfactory for this purpose.

[12] Cell-Tak BD Biosciences, San Jose, CA, has been found satisfactory for this purpose.

processing serum lubricants). Isolate the metal or ceramic particles following the metal protocol described in 11.2.10 and following. In case there are PE particles mixed with metal or ceramic particles, or both, after isolating the latter on a Si wafer, treat the supernatant according to the PE protocol described in 11.1.

11.4 *Procedure for Digestion of Serum Proteins for Harvesting Particles from Synovial Fluid*

Sample Handling

11.4.1 Handling of synovial fluid samples should be performed according to Guide F2995.

Digestion Protocol

11.4.2 Mix 150 µL of 1M HEPES (pH=7.5), 6 µL of 1 M MgCl2, 75 µL of 0.5 M CaCl2, and 2.8mL of synovial fluid for a total of 3 mL in a 50 mL siliconized blue capped tube. (In less than 2.8 mL of synovial fluid are available, add deionized water to a total volume of 2.8 mL.) Add 300 µL of dilute Hyaluronidase (0.05 % (g/100 mL) Hyaluronidase, 0.1 M NaH2PO4 (pH 5.3), 0.15M NaCl) to the synovial fluid mix.

11.4.3 Incubate at 37ºC with a gyration of 250 rpm for 6 hours.

11.4.4 Add 5µl of diluted Benzonase (that is, 5 µL of Benzonase in 50 µL of: 50 % glycerol, 0.02 M Tris HCl (pH=8.0), 0.002 M MgCl2, 0.02 M NaCl) to each sample.

11.4.5 Incubate at 37ºC with a gyration of 250 rpm overnight (total fluid 3.305 mL).

11.4.6 Follow the protocol established for the digestion and separation of the metal/ceramic particles (11.2.6 and following) Since synovial fluid does not contain EDTA as in simulator lubricant, the amount of calcium to be added should lead to 18 mmol/l rather than 40mmol/l as per the simulator extraction. In case there is a need for recovering or characterizing PE particles, first collect the metal or ceramic particles, or both, on a Si wafer and then treat the supernatant as described for the PE protocol (11.1.11 and following). In case there are no metal/ceramic particles or there is no interest in characterizing them, follow directly the protocol for the isolation of PE particles (11.1.11 and following).

11.5 *Morphologic Characterization*

11.5.1 Perform morphologic characterization using digital image processing software, or manually.

11.5.2 Treat images to reduce noise and increase the definition of particles against the background.

11.5.3 Convert the images to black and white.

11.5.4 Outline the particles on each micrograph.

11.5.5 Particle morphology can be characterized using Practice F1877. Alternatively, the following characteristics can be measured: width (W), height (H), length (dmax), breadth (dmin), fiber length (FL), fiber breadth (FB), perimeter (P), and area (A), and these data can then used to calculate the five morphologic parameters specified by Practice F1877 [6], that is, equivalent circle diameter (ECD), aspect ratio (AR), elongation (E), roundness (R), and form factor (FF).

11.5.6 The ratio between E and FF is used for basic identification and classification of the shape of each particle. Specifically, particles with an E/FF value up to 1.2 are considered round, those between 1.2 and 5.4 oval, those between 5.4 and 150 rod like or irregular in shape, and those higher than 150 fibril-shaped.

11.5.7 To further distinguish rod like from irregular particles (in the group with E/FF values between 5.4 and 150), use the value obtained through Elliptical Fourier Analysis (EFA3).

11.5.8 After separating the rods and irregulars from other particles using E/FF as outlined above, any particle with an EFA3 greater than 20 are considered irregular.

11.5.9 If the EFA3 value is 20 or less and if d_{max}/FL is less than 0.8, the particle is classified as irregular.

11.5.10 If the EFA3 value is 20 or greater and d_{max}/FL is 0.8 or greater, the particle is classified as a rod.

12. Isolation of Debris

12.1 The debris obtained by the above procedures may contain particles of a wide range of sizes and morphology. There may also be clumping or agglomeration of particles. As a result, several stages of filtration may be necessary to effectively isolate the different particles of interest.

12.2 The final common pathway for the above procedures is filtration with submicron filters. These are then subjected to optical or scanning electron microscopy, or particle counting. Particle morphology may be characterized.

12.3 To characterize retrieved particles with light microscopy, the particle solution should be sonicated for 10 min. Some particles may melt and agglomerate further by sonication for 10 min. For these particle systems, short bursts with time to cool are recommended. From this sonicated solution, 10 µl of the solution can be removed and filtered using a 0.1 µm filter (nitrocellulose, Millipore, Nucleopore). The filter should then be mounted and coverslipped on a glass slide. Since the filters become optically transparent when in contact with standard mounting media, the particles can be analyzed with a transmitted light microscope.

12.4 To characterize the morphology of retrieved particles with scanning electron microscopy, 10–200 µl of particle solution should be filtered using a 0.2 µm polycarbonate filter, or 10 and 0.4 µm filters (nitrocellulose, Millipore, Nucleopore). The filtering vacuum should be stopped before all the liquid is removed, and the remaining liquid is allowed to evaporate. The filter should then be fixed with double-sided tape and copper conductive strip on an SEM mount. Specimens may be coated with palladium or gold to make the polymeric particles conductive. Coating is not necessary when operating the SEM in the low vacuum environmental mode.

12.5 The large pore filters can be cut in half, dried at 50°C and coverslipped for viewing with transmitted polarized light microscopy.

12.6 To characterize particles in the nanometer size range, filtration should be performed with successive filtration steps. Larger debris is removed by sequential filtration from 10 to 0.1 µm cyclopore membranes (Whatman), or 0.03 and 0.01 micron polycarbonate track-etched (Millipore, Nucleopore & Osmonics/Poretics) PCTE filters. These filters will often capture nanoparticles due to their strong susceptibility to Brownian motion. Finer filtration can be achieved with sintered ceramic filters for separation of viruses and bacteria. These

filters can have pore sizes on the order of 25 nm. For scanning electron microscopy (SEM) analysis, the filter should be mounted on an SEM stub with double-sided carbon tape and coated with 100-200 Å of gold or platinum. Standard high vacuum SEM can then be conducted. SEM is usually used for particle sizes from 50 nm to 1 cm. For higher resolution transmission electron microscopy (TEM), the particles in the filter can be resuspended in isopropanol and then pipeted onto holey carbon films. After air-drying, TEM can be conducted on the films. TEM is usually used for particle sizes from 5 nm to 500 µm.

12.7 Particle morphology should be characterized using Practice F1877 as a guide.

12.8 Chemical analysis methods such as EDXA and FTIR may also be employed to determine the chemical nature of the particles.

13. Stage I Analysis of the Implant Components

13.1 Stage I examination and documentation should be conducted routinely on all retrieved devices. The procedures are intended to take the investigator a minimal amount of time and expense to complete, while providing sufficient information to permit device tracking.

13.1.1 Perform a separate analysis for each component of a device, if possible and necessary, as outlined below. A coating shall be considered as a separate component. A sample form is provided as Appendix X2.

13.1.2 Document chain of device custody as per Practice E1188.

13.2 *Device Description:*

13.2.1 Identify the device type and describe all of the components retrieved.

13.2.2 Record the manufacturer and catalogue and serial numbers where possible.

13.2.3 Record any other information which describes the device.

13.3 *Macroscopic Examination:*

13.3.1 Perform this examination with the unaided eye or with the aid of a stereo-microscope.

13.3.2 Examine all surfaces of the components for evidence of in service or iatrogenic damage.

13.3.3 Record an estimate as to the degree of findings as per Appendix X2.

13.4 *Visual Examination for Evidence of Mechanical Failure:*

13.4.1 Observe the implant surface by suitable techniques to ascertain any mode of failure, as applicable. In no event should any surface of a failed implant be destructively evaluated at this time.

13.4.2 Sketch any significant features. These may also be recorded photographically, although this adds significant cost to routine, Stage I analysis.

14. Stage II Analysis

14.1 Stage II analysis should be carried out after Stage I, if deemed necessary, to further evaluate the characteristics, or failure mode, of the implant. This level of testing primarily relates to an assessment of the modes of failure and deterioration of an implant in the most non-destructive manner possible. Record the results on form Appendix X3.

14.1.1 Perform Stage II analysis for severely damaged components, or where optical fractography is sufficient to determine mechanisms involved in mechanical failure of the device.

14.1.2 Fabrication of surface replications may be indicated in some Stage II investigations. These could then be used for examination without damaging the specimen.

14.2 *Photography:*

14.2.1 A photographic documentation of findings should be made.

14.3 *Optical Fractographic Examination:*

14.3.1 If the implant is fractured, analyze the fracture surface by suitable techniques to ascertain the mode of fracture.

14.3.2 In no event should the fracture surface be destructively evaluated. If the device has mechanically failed, it is important to remember that it may be classified as legal evidence.

15. Stage III Analysis, Metallic Components

15.1 If further testing is necessary to assess the properties of the implant, the tests listed under Stage III Analysis, Metallic Components, in Appendix X4 shall be carried out as deemed necessary to further characterize the implant and its history.

15.1.1 Stage III examinations involve destructive methods for microscopy and chemical analysis. These should only be conducted when complete material characterization and failure analysis is indicated. Care should be taken to insure that potential legal evidence is not destroyed, as per Practice E860.

15.2 *Microscopic Examination:*

15.2.1 Prepare appropriate metallographic sections in accordance with Methods E3 and Terminology E7.

15.2.2 Specimens should be etched as per Practice E407, and examined microscopically using light microscopy as per Guide E883.

15.2.3 Determine the inclusion content using Test Methods E45 as a guide. Compare the observed content with that of the applicable material standard, if appropriate.

15.2.4 Determine the grain size as per Test Methods E112 and compare results with the applicable material standard.

15.2.5 Characterize the grain boundaries in terms of inclusion content. Microscopic evidence of corrosion should be noted and recorded. Evidence of sensitization of stainless steels can be determined according to Practices A262.

15.2.6 Any evidence of microporosity can be documented using Test Methods E45 as a guideline.

15.2.7 The use of scanning electron microscopy as per Practice E986 may be utilized for fractographic analysis.

15.3 *Material Characterization:*

15.3.1 Determine the physical and chemical composition and identity of the metallic alloy as per Test Methods A751, E353, E354, and E120. In the event that the composition does not meet a recognized material standard, the appropriate referee analysis procedure shall be used.

15.3.2 Chemical analysis can also be conducted with electron microprobe analysis.

15.4 *Mechanical Properties:*

15.4.1 The type of measurements to be carried out at this Stage of characterization will be dependent upon the implant and its use. Suggested property tests are shown in Appendix X4 under Mechanical Properties.

15.4.2 Determine the hardness as per Test Methods E10, E18, or E92.

15.4.3 Determine the tensile, flexural, compressive, and the like properties in accordance with applicable material specification if indicated, and such other tests as are appropriate to the specimen which may be fabricated from the implant. Deviation from the specimen dimensions as described in the standard methods may be necessary to accommodate the shape and size of the device under investigation.

15.5 *Coated Samples:*

15.5.1 Examine coated samples microscopically for missing sections of coating. Estimate the fraction of coating missing. Coating thickness and void content may be determined as per Test Method F1854.

15.5.2 Where possible determine the shear strength of the adherent coating as per Test Method F1044.

15.5.3 Where possible determine the tensile strength of the adherent coating as per Test Method F1147.

16. Stage III Analysis: Polymeric Components

16.1 If further testing is necessary to assess the properties of the implant, the tests listed under "Stage III Analysis, Polymeric Components," in Appendix X5 shall be carried out as deemed necessary to further characterize the implant and its history.

16.1.1 Stage III examinations involve destructive methods for microscopy and chemical analysis. These should only be conducted when complete material characterization and failure analysis is indicated. Care should be taken to insure that potential legal evidence is not destroyed, as per E860.

16.1.2 A wide variety of polymeric materials are used in implantable devices. Actual identification of the material type may not be possible without Stage III chemical analysis. Where possible, classify the material according to Classification D4000.

16.1.3 Each type or class of materials has an associated battery of tests designed for that specific type. As a result, the investigator is directed to the specific material standards to identify the specific tests which are appropriate. The methods and properties described in this section are intended to serve as a guide to the selection of test methods.

16.2 *Microscopic Examination:*

16.2.1 Examine the specimen for evidence of surface damage or degradation.

16.2.2 The use of thin sections (5–10 μm) and optical microscopy using reflected and transmitted, polarized and nonpolarized light has been found useful in identifying evidence of inclusions or porosity and for identification of changes in crystallinity or density.

16.2.3 In the case of mechanical failure, scanning electron microscopy (SEM) may provide insight into the mode of failure.

16.3 *Material Characterization*

16.3.1 Determine the molecular weight or weight distribution of the specimen. Possible methods and ASTM standards to be utilized are gel permeation chromatography (GPC) (Practice D3016, Test Method D5296), osmometry, light scattering (Test Method D4001), viscometry (Test Methods D3835, D2857), or melt index (Test Method D1238).

16.3.2 Determine the density by an appropriate method. Suggested methods are by displacement (Test Methods D792), the gradient method (Test Method D1505), or, for rigid cellular plastics, Test Method D1622 is suggested.

16.3.3 Determine the thermal properties of the material. The glass transition (Tg), and melt temperature (Tm) can be determined by differential scanning calorimetry (DSC) as per Test Method D3417. Phase identification by differential thermal analysis (DTA) as per Test Method D3418. Softening point, thermomechanical properties, coefficient of expansion can be determined by thermomechanical analysis (TMA). The thermal stability can be determined by thermogravimetric analysis (TGA).

16.3.4 Determine the chemical composition and determine whether there has been any oxidation or degradation of the material. Analysis by infrared analysis and FTIR is suggested, as per Practices E168 and E204. Chemical composition and structure can be determined by nuclear magnetic resonance spectroscopy (NMR) as per Practice E386. Free radicals can be identified with electronic spin resonance (ESR).

16.3.5 Of particular interest are the presence of any low molecular weight extractables. These may be from degradation of the polymer or from absorption of moieties such as lipids from the *in vivo* environment. There are a number of extraction protocols that could be utilized, for example, Test Methods D1239, D2842, D4754, Practice D5152, Test Method D5227, Practice F619. Appropriate chemical analysis assays can then be applied to identify the composition of the etractables. The results will depend on the method chosen.

16.4 *Mechanical Properties:*

16.4.1 The type of measurements to be carried out at this Stage of characterization will be dependent upon the implant and its use. In making such measurements, it is important to note and record the sample location and orientation.

16.4.2 Determine the hardness according to the applicable material standard. Testing by Rockwell, as per Test Method D785, or Durometer according to Test Method D2240 are suggested.

16.4.3 Determine the tensile, flexural, compressive, etc. properties in accordance with applicable material specification if possible, and such other tests as are appropriate to the specimen which may be fabricated from the implant. Suggested test methods are: tensile (Test Methods D412, D638, D1623, D1708), flexural (Test Methods D621, D671, D747, D790), compression (Test Methods D695, D1621), shear (Test Method D732), impact resistance (Test Methods D256), tear resistance (Test Methods D624, D1004), abrasion resistance (Test Method D1242), and tensile creep (Test Method D2990).

Mechanical properties of UHMWPE specimens may also be determined with the small punch method in Test Method F2182. Deviation from the specimen dimensions as described in the standard methods may be necessary to accommodate the shape and size of the device under investigation.

16.4.4 Dynamic mechanical properties may also be of interest. These could be measured by means of a torsional pendulum as per Test Method D4065 or in flexural fatigue as per Test Method D671.

16.4.5 The extent of oxidation of UHMWPE specimens may be determined as per Guide F2102.

16.4.6 Network parameters of crosslinked UHMWPE may be determined as per Test Method F2214.

16.5 *Coated or Porous Specimens:*

16.5.1 Coatings should be microscopically and chemically characterized utilizing the methods as described in this section.

16.5.2 The porosity of porous materials or porous coatings should be determined according to Test Method F1854, Test Method D2873, Practice E562 or Test Method F316.

16.6 *Polymer Matrix Composites:*

16.6.1 Analysis and characterization of polymer matrix should be as described in this section.

16.6.2 Analysis of the reinforcing material should be in accordance with the material type.

16.6.3 Of special interest with composites is the analysis of the interfacial relationships between matrix and the second phase. Microscopic methods should be utilized to establish degrees and nature of interfacial bonding, or degradation thereof.

17. Stage III Analysis: Ceramic and Glass Materials

17.1 If further testing is necessary to assess the properties of the implant, the tests listed under "Stage III Analysis: Ceramic and Glass Materials" In Appendix X6 shall be carried out as deemed necessary to further characterize the implant and its history.

17.1.1 Stage III examinations involve destructive methods for microscopy and chemical analysis. These should only be conducted when complete material characterization and failure analysis is indicated. Care should be taken to insure that potential legal evidence is not destroyed, as per Practice E860.

17.1.2 There is a wide variety of materials and devices that fit this category. Therefore, these protocols shall serve as a guide to ascertaining the important features of retrieved devices.

17.2 *Microscopic Examination:*

17.2.1 Use standard light optical or electron optical microscopic preparation techniques suitable for the material under investigation.

17.2.2 Determine the inclusion content in accordance with the applicable material standard, if appropriate.

17.2.3 Determine the grain size in accordance with the applicable material standard and method.

17.2.4 Evidence of degradation should be noted and recorded as per Appendix X6. Surfaces should be examined for evidence of surface etching or grain excavation.

17.2.5 Fracture surfaces may be examined by optical and scanning electron microscopy (SEM) as per Practice C1322.

17.3 *Material Characterization:*

17.3.1 Determine the chemical composition and identity of the component. Chemical composition of glasses may be conducted according to Test Methods C169. Composition of ceramics may be conducted according to Test Methods C573.

17.3.2 Determine the density and apparent porosity, as per Test Methods C20.

17.3.3 Appropriate x-ray diffraction (XRD) methods should be utilized to determine degree of crystallinity and phase composition. Phase composition may also be determined by FTIR.

17.3.4 Thermal properties may be determined by differential scanning calorimetry (DSC), or thermogravimetric analysis (TGA) as appropriate, and by differential thermal analysis (DTA).

17.4 *Mechanical Properties:*

17.4.1 The type of measurements to be carried out at this Stage of characterization will be dependent upon the implant and its use. Suggested property tests are shown in Appendix X6 under Mechanical Properties.

17.4.2 Determine the hardness as per Test Method C730 for glasses or Test Methods C1326 or C1327 for ceramics.

17.4.3 Determine the tensile, flexural, compressive, etc. properties in accordance with applicable material specification, if possible. Modulus and Poisson's ratio may be determined as per Test Method C623 for glasses, or Test Method C1198 for ceramics. Flexural properties may be determined as per Test Methods C158 for glasses, and Test Methods C674 or C1161 for ceramics.

17.5 *Porous and Coated Samples:*

17.5.1 Examine coated samples microscopically for missing sections of coating. Estimate the fraction of coating missing.

17.5.2 Determine the shear strength of the coating using the appropriate method, such as Test Method F1044.

17.5.3 Determine the tensile strength of the coating using the appropriate method, such as Test Method F1147.

17.5.4 Determine adhesion or cohesive strength of flame-sprayed coatings as per Test Method C633.

17.5.5 Surface area of porous materials may be determined by Test Method C1069. Porosity may be determined as per Test Method F1854, Practice E562, or Test Method F316.

17.6 *Ceramic Matrix Composites:*

17.6.1 Analysis and characterization of ceramic matrix should be as described in this section.

17.6.2 Analysis of the reinforcing material should be in accordance with the material type.

17.6.3 Of special interest with composites is the analysis of the interfacial relationships between matrix and the second phase. Microscopic methods should be utilized to establish degrees and nature of interfacial bonding, or degradation thereof.

18. Materials of Biological Origin or Tissue Engineering

18.1 If further testing is necessary to assess the properties of biologically derived implants, the tests shall be carried out as deemed necessary to further characterize the implant and its history.

18.1.1 Stage III examinations involve destructive methods for microscopy and chemical analysis. These should only be conducted when complete material characterization and failure analysis is indicated. Care should be taken to insure that potential legal evidence is not destroyed, as per Practice E860.

18.2 The protocols in this document provide for analysis of biological tissues and of synthetic materials. The application of the appropriate methodologies should be utilized in the study of various biologic and synthetic components of retrieved biological or tissue engineered devices.

19. Keywords

19.1 ceramics; chemical analysis; composites; histology; implant retrieval; metals; particles; polymers

APPENDIXES

(Nonmandatory Information)

X1. CLINICAL INFORMATION SUGGESTED TO BE RECORDED DURING RETRIEVAL OF MEDICAL DEVICES

Minimum data set
Date of implantation, and date of removal
Hospital, or physician's office of implantation and of removal
Confidential patient ID Code to link to hospitals of implan- tation and removal records
Implant anatomical site
Device identification (manufacturer's name and device cata- logue number), Device lot and serial number
Indication for use and Reason for explantation
Additional Clinical Information (confidential)
Surgeon (name & address)
Patient (name & address and/or ID number)
female [ ] male [ ] date of birth ____ occupation; weight height
History of substance abuse (smoking, etc.)
Reason for Investigation: routine series [ ], research [ ] documentation [ ], complaint [ ], liability claims [ ], clinical investigation [ ], other _____
Diagnosis at Insertion (or Reason for Insertion)
Additional Diagnoses and Complications
Antibiotics: pre op. [ ] peri op. [ ] post op. [ ] prophylactic [ ]
Relevant pharmaceuticals duration:
Post Operative Treatment:
Complications between insertion and removal: e.g. infection [ ]
Observations prior to removal (functional)
Clinical Reason for Removal:
routine [ ] pain [ ] revision [ ] failure [ ] infection [ ]
allergy [ ] other _____
Patient level of activity at removal
Observations at Removal (indicate yes, no, not applicable, doubt, etc.)

normal tissue [ ]	bursal fluid [ ]	scar tissue [ ]
granulation tissue [ ]	bone reaction [ ]	infection [ ]
loose implant [ ]	discoloration, implant debris [ ]	other _____

Additional Material Provided for Analysis

radiographs	no [ ]	yes [ ]	how many
tissue	no [ ]	yes [ ]	type origin
bacteriol. specimen	no [ ]	yes [ ]	type origin
immunol. specimen	no [ ]	yes [ ]	type origin
fluid	no [ ]	yes [ ]	type

photographs; pathology reports
surgical reports; additional documentation
Type of disinfection used

X2. STANDARD FORM FOR GUIDING THE STAGE I ANALYSIS OF RETRIEVED IMPLANTABLE MEDICAL DEVICES

Reason for Investigation:
routine clinical series [ ] animal study [ ] research [ ] complaint [ ] liability claims [ ] other ____
Patient or animal ID code _____
Date Retrieved _____ Implant duration _____
Date of analysis _____
Record for all Components retrieved:
device type & component names _____
manufacturer(s) _____
catalog number(s) _____ Serial number(s) _____
identification marks, e.g. size: _____
material(s) _____
Macroscopic examination (YES, NO, DOUBT, or NOT APPLICABLE)

		Location	Size/Area	Severity/ Degree
_____ a)	wear or burnishing	_____	_____	_____
_____ b)	galling	_____	_____	_____
_____ c)	scratching	_____	_____	_____
_____ d)	change of shape	_____	_____	_____
_____ e)	mechanical damage	_____	_____	_____
_____ f)	macro porosity	_____	_____	_____
_____ g)	pitting or crevice corrosion	_____	_____	_____
_____ h)	fretting	_____	_____	_____
_____ i)	embedded particles	_____	_____	_____
_____ j)	discoloration or staining	_____	_____	_____
_____ k)	calcification	_____	_____	_____
_____ l)	thrombosis	_____	_____	_____
_____ m)	degradation	_____	_____	_____
_____ n)	stress cracking or crazing	_____	_____	_____
_____ o)	loss of coating	_____	_____	_____
_____ p)	mechanical failure	_____	_____	_____
_____ q)	cold flow	_____	_____	_____

Sketch(s):

X3. STANDARD FORM FOR GUIDING THE STAGE II ANALYSIS OF RETRIEVED IMPLANTABLE MEDICAL DEVICES

Photograph orientation and descriptions.

Mechanical failure (if YES, identify mode, indicate location of failure and method of identification)

a) static-overstress, causing plastic deformation
b) shear
c) fatigue
d) torsion
e) impact
f) stress corrosion or environmental cracking
g) fatigue, or corrosion-fatigue
h) combination of above (identify)
i) other (specify)_____
j) unable to identify

X4. STAGE III ANALYSIS: METALLIC MATERIALS

Standard form for guiding the Stage III analysis of retrieved metallic implantable medical devices

1. **Microscopic examination** (indicate location and orientation of sample)
a) Inclusion content
b) Grain size
c) Grain boundary constituents
d) Microporosity
e) Other distinguishing features
2. **Failure analysis** (if appropriate)
a) Presence of fatigue striations
b) Fraction of surface with ductile overload
c) Evidence of significant ductile overload
d) Defects associated with crack initiation
3. **Type of material** (indicate method of determination)
a) Chemical composition
4. **Mechanical properties** (indicate N/A if not available). Samples should be taken from areas representative of the original material.
a) Sample size and orientation
b) Hardness (indicate type and method)
c) Other ASTM tests as applicable (for example, tensile, or transverse bend tests)
5. **Metallic Coating**
a) Coating material
b) Estimated fraction of coating missing
c) Shear strength
d) Tensile strength

X5. STAGE III ANALYSIS: POLYMERIC MATERIALS

Standard form for guiding the Stage III analysis of retrieved polymeric components

Samples should be taken from areas representative of the original material, (D1898).

1. **Microscopic examination** (indicate location and orientation of sample)
a) Grain size
b) Grain boundary constituents
c) Microporosity
d) Evidence of damage or degradation, or other distinguishing features.
2. **Failure analysis:**

Mode of failure, delamination, oxidation, optical microscopy with polarized light.

3. **Material Characterization**
a) Molecular weight (use most applicable technique)
1) Gel permeation chromatography (GPC)
2) Osmometry
3) Light scattering
4) Viscometry
5) Melt index
b) Density
c) Thermal characterization (most appropriate techniques)
1) Glass transition (Tg), melt temperature (Tm), by differential scanning calorimetry (DSC). Phase identification by differential thermal analysis (DTA).
2) Softening point, thermomechanical properties, coefficient of expansion by thermomechanical analysis (TMA)
3) Thermal stability by thermogravimetric analysis (TGA)
d) Chemical analysis
1) Infrared spectroscopy (IR, FTIR)
2) Chemical composition and structure (NMR)
3) Other chemical composition determinations
e) Low molecular extractables, e.g. absorbed lipid content of implant.
4. **Mechanical properties** (indicate N/A if not available).
a) Record sample size and orientation
b) Hardness
c) Other tests as appropriate, e.g. tensile, flexural, compression, shear, impact resistance, tear resistance, abrasion resistance, tensile creep.
d) Dynamic mechanical measurements, fatigue.
5. **Porous or Coating materials**
a) Coating material
b) Any damage of loss of coating
c) Porosity
6. **Composites**
a) Type of composite materials
b) Matrix phase relationships.

X6. STAGE III ANALYSIS: CERAMIC AND GLASS MATERIALS

STANDARD FORM FOR GUIDING THE STAGE III ANALYSIS OF RETRIEVED CERAMIC AND GLASS IMPLANTABLE MEDICAL DEVICES

1. **Microscopic examination** (indicate location and orientation of sample)
   a) Inclusion content
   b) Grain size
   c) Grain boundary constituents
   d) Microporosity
   e) Grain excavation, grain orientation, (texturing) of surface vs bulk
   f) Surface morphology (SEM)
   g) Other distinguishing features
2. **Failure Analysis** (if appropriate)
   a) Defects associated with crack initiation
   b) Fracture morphology indicative of failure stresses
3. **Material Characterization**
   a) Type of material (indicate method of determination)
   b) Chemical composition
   c) Surface chemistry, etching, new crystals
   d) Chemical glass transition (DSC or DTA)
   e) Crystallinity (XRD)
   f) Phase composition (FTIR)
4. **Mechanical properties** (indicate N/A if not available). Samples should be taken from areas representative of the original material.
   a) Sample size and orientation
   b) Hardness (indicate type and method)
   c) Other ASTM tests as applicable (for example, flexural properties)
5. **Porous or Ceramic Coatings**
   a) Coating material
   b) Estimated fraction of coating missing
   c) Shear strength
   d) Tensile strength
   e) Adhesive and cohesive strength
6. **Composite Materials**
   a) Material types
   b) Composite phase relationships

X7. RATIONALE

X7.1 The ultimate test for an implant or device and the materials out of which is fabricated, is its performance as an implant. In many situations, critical information can only be ascertained by examination of retrieved implants and the surrounding tissues. Such analysis could be part of experimental studies in animals, clinical studies, where devices are routinely removed or removed for revision, or in post-mortem autopsy studies. The information suggested for collection could also be of value in device tracking.

X7.2 This practice was first published in 1978 for analysis of retrieved metallic orthopaedic implants. Rather than develop a separate standard for each material type or class, the document was revised to cover all material types and analysis of the associated tissues.

X7.3 The intent of this practice is to provide standard protocols for analysis of retrieved devices. It specifies standard methods of analysis and data collection for comparing similar sets of data between research centers. For information and data on retrieved devices to be useful on a national and international basis, standard methods of analysis and reporting are necessary. Therefore, collected data should conform to this standard practice.

X7.4 Because of the complexity of analysis of implant devices, and because of the large number of potential tests suggested in this practice, it has appeared desirable to divide the investigation into stages. The analyses of the implant are separated into three groups with the degree of characterization increasing from Stage I through Stage III. The implant characterizations may include macroscopic and microscopic examinations, chemical composition, as well as physical and mechanical property determinations to ascertain mechanisms and degree of degradation.

X7.5 The tests performed may depend upon the reason for removal of the implant. Furthermore, analysis can be an expensive and time consuming process. To facilitate capturing information on a large number of implants, a minimum dataset obtained by inexpensive means is proposed in the first two stages. Perform a Stage I analysis for all removals. Perform a Stage II analysis for severely damaged components, or where optical fractography is sufficient to determine mechanisms involved in mechanical failure of the device. Stage III examinations involve destructive methods for microscopy and chemical analysis. These should only be conducted when complete material characterization and failure analysis is indicated.

X7.6 The American Society for Metals, International, has published Metals Handbook, Failure Analysis and Prevention, which may be used as a reference to supplement the practices in this document. As part of an Implant retrieval meeting held at the National Bureau of Standards in 1980, an extensive collection of retrieval forms was assembled as an appendix,[13] which may also be used as a guide for documenting the analyses.

X7.7 This practice is intended for clinical and other research purposes. Users interested in product liability litigation may refer to E860. In any event, care should be taken to insure that potential legal evidence is not destroyed.

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