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Standard Guide for Testing the Biological Responses to Medical Device Particulate Debris and Degradation Products *in vivo*[1]

This standard is issued under the fixed designation F1904; 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 The purpose of this standard guide is to describe the principles and approaches to testing of medical device debris and degradation products from device materials (for example, particles from wear) for their potential to activate a cascade of biological responses at local and systemic levels in the body. In order to ascertain the role of device debris and degradation products in stimulating such responses, the nature of the responses and the consequences of the responses should be evaluated. This is an emerging area. The continuously updated information gained from the testing results and related published literature is necessary to improve the study designs, as well as predictive value and interpretation of the test results regarding debris/degradation product related responses. Some of the procedures listed here may, on further testing, not prove to be predictive of clinical responses to device-related debris and degradation products. However, only the continuing use of standard protocols will establish the most useful testing approaches with reliable study endpoints and measurement techniques. Since there are many possible and established ways of determining the debris/degradation product related responses *in vivo*, a single standard protocol is not stated. However, this recommended guide indicates which testing approaches are most applicable per expected biological responses and which necessary information should be supplied with the test results. To address the general role of chronic inflammation in exaggerating device-related foreign body response (FBR), the recommendations in this standard include the assessment of device-related pro-inflammatory responses and subsequent tissue remodeling potential.

1.2 This document is to provide the users with updated scientific knowledge that may help better characterize medical device debris related responses. It is to help the users to optimize their plans for particle characterization and biocompatibility assessment by considering the testing principles and methods available in published literature that are appropriate to their products.

1.3 This standard is not sufficient to address device-related degradation products that result in gas formation or that are exclusively represented by nanoparticles, or soluble species such as dissolved metal ions.

1.4 While devices should be designed and manufactured in such a way as to reduce as far as possible the risks posed by substances or particles (including wear debris, degradation products, and processing residues) that may be released from the device, this standard guide may help users to identify the presence of wear debris and degradation products and subsequent adverse reactions that may occur.

1.5 Although this guide is based on the available device debris-related knowledge that is largely based on orthopedic devices, most of the recommendations are also applicable to other (non-orthopedic) device areas.

1.6 *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, health, and environmental practices and determine the applicability of regulatory limitations prior to use.*

1.7 *This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the Development of International Standards, Guides and Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.*

2. Referenced Documents

2.1 *ASTM Standards:*[2]

F561 Practice for Retrieval and Analysis of Medical Devices, and Associated Tissues and Fluids

F619 Practice for Extraction of Materials Used in Medical Devices

[1] This guide is under the jurisdiction of ASTM Committee F04 on Medical and Surgical Materials and Devices and is the direct responsibility of Subcommittee F04.16 on Biocompatibility Test Methods.

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[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.

F748 Practice for Selecting Generic Biological Test Methods for Materials and Devices
F1877 Practice for Characterization of Particles
F1903 Practice for Testing for Cellular Responses to Particles ***in vitro***

2.2 *ISO Standards:*[3]
ISO 14242-1 Implants for surgery—Wear of total hip-joint prostheses—Part 1: Loading and displacement parameters for wear-testing machines and corresponding environmental conditions for test—Amendment 1
ISO 14242-3 Implants for surgery—Wear of total hip-joint prostheses—Part 3: Loading and displacement parameters for orbital bearing type wear testing machines and corresponding environmental conditions for test
ISO 14243-1 Implants for surgery—Wear of total knee-joint prostheses—Part 1: Loading and displacement parameters for wear-testing machines with load control and corresponding environmental conditions for test
ISO 14243-3 Implants for surgery—Wear of total knee-joint prostheses—Part 3: Loading and displacement parameters for wear-testing machines with displacement control and corresponding environmental conditions for test
ISO 17853 Wear of implant materials—Polymer and metal wear particles—Isolation and characterization
ISO 22622 Implants for surgery—Wear of total ankle-joint prostheses—Loading and displacement parameters for wear-testing machines with load or displacement control and corresponding environmental conditions for test

3. Terminology

3.1 *Definitions of Terms Specific to This Standard:*

3.1.1 *mechanistic, adj*—of or relating to the theory of mechanism which, in the science of biology, is defined as a system of causally interacting parts and processes that produce one or more effects.

3.1.2 *phagocytosable, adj*—capable of being phagocytosed.

4. Summary of Guide

4.1 Evaluation of biological responses to medical device debris and degradation products may be performed using specimens from animals being tested in accordance with Practice F748 which provides recommendations for biocompatibility assessment including local and systemic toxicity. When biocompatibility testing is performed (for example, implantation or injection of the test material), evaluation of the tissues surrounding the application site represent the best opportunity for assessing FBR and other local tissue responses. Bodily fluids such as blood and urine, as well as different organ tissues from the tested animals should be used for the assessment of systemic responses. Procedures according to Practice F561 may be used to assess the cellular and tissue responses *in vivo*.

4.2 Biological responses to device-related wear debris and degradation products may be tested using materials or extracts in accordance with Practice F619. The increased surface area of small particles may enhance the amount of extracted substances but, since the response to particles may be related to the physical size, shape, composition, and dose, the use of only extracts will not completely address the question of the impact of particle formation on the tissue response, and actual implantation or other testing of particles should be included as a part of the characterization of tissue response when particle generation is likely during actual usage. These materials or extracts may be used for the *in vivo* tests described here or *ex vivo* / *in vitro* approaches described in Practice F1903. Particles and other device-related debris/degradation products generated by alternative methods (for example, from animal studies, clinical use, or *in vitro* studies) may also be used, if appropriately justified. The method of generation must be described.

5. Significance and Use

5.1 This standard guide is to be used to help assess the biocompatibility of materials used in medical devices (for example, externally communicating, implants, and other body contact medical devices). It is designed to test the effect of particles and other wear debris and/or degradation products on the generation of FBR and other (local and systemic) host responses of immune/inflammatory origin.

5.2 The appropriateness of the selected testing methods should be carefully considered by the user since not all materials or applications need to be tested by this guide. Existing biocompatibility screening methods may not be fully predictive of the human response, and testing approaches such as those described here are needed for continuous improvement of the predictability of biocompatibility testing. The effectiveness of animal testing in terms of its predictability of human outcomes is dependent on the study design. If possible, study endpoints should be chosen to minimize interspecies variability and to investigate clinically relevant biological responses. While testing approaches should remain at the user's discretion, the following should be taken into consideration when selecting most appropriate tests and study endpoints.

5.2.1 Device-induced responses usually involve both innate and adaptive immunities, which raises possible need for specific testing for each of these immune response types.

5.2.1.1 Device-related adaptive immune responses are mostly due to lymphocyte-mediated delayed-type hypersensitivity. *In vivo* allergenicity to a test material (which can be introduced via different routes) should be assessed by monitoring for any signs of allergic and acute toxicity reactions, for example, scratch, tremor, and dyspnea. In addition, *ex vivo* analysis on immunophenotyping of the isolated splenocytes/lymphocytes from the same studies should be considered.

5.2.1.2 Device-related innate immune responses are mostly mediated by macrophages and can be assessed by histopathological assessment of the extent of FBR including macrophage accumulation around the test material. Supplementary *ex vivo* / *in vitro* assessment can be used for additional macrophage-based testing such as macrophage immunophenotyping (proinflammatory M1 and anti-inflammatory/wound healing M2) as well as debris uptake by phagocytes (phagocytozability) involving the entire range of test material characteristics.

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

5.2.2 Due to the role of inflammation in extending device-related FBR and promoting the resultant tissue remodeling, histopathological assessment should include identification of immune/inflammatory cell infiltration (with separate counts for the individual cell types representing both innate and adaptive responses) as well as corresponding tissue changes (for example, fibrosis, necrosis, ossification or osteolysis, angiogenesis). Identification of immune/inflammatory cells may involve different approaches including IHC phenotyping as needed. Supplementary *ex vivo* / *in vitro* assessment should be considered for assessing the balance in release of pro-inflammatory versus anti-inflammatory cytokines as well as generation of hyper-proliferative versus hypo-proliferative tissue responses.

5.2.2.1 Since the signs of inflammation and post-inflammatory tissue changes may not be always apparent, special attention should be given to the assessment of debris-related inflammogenic and tissue remodeling potentials using *ex vivo* specimens and supplementary *in vitro* assessment when needed. Pro-inflammatory cell death (necrosis) should be distinguished from programmed cell death (apoptosis usually associated with anti-inflammatory responses) by using cell viability and cytotoxicity testing involving cellular staining and flow cytometry. Given the importance of phagocytes in proper clearance of dying cells, normal non-phlogistic phagocytosis of cells undergoing apoptosis should be distinguished from "frustrated" phlogistic phagocytosis which may result in further cell/tissue damage due to the release of damage-associated molecular patterns (DAMP). See X1.10 for more details.

5.2.3 Due to the role of the device-tissue interface in shaping biological responses, *in vivo* models as well as supplementary testing should be aimed to simulate (as much as possible) device-specific use environments. *In vivo* animal models with intra-articular applications of a test material may be beneficial for testing of orthopedic materials, while intracardiac/intravenous applications may be more beneficial for testing of cardio/endovascular materials.

5.2.3.1 Since many implantable materials come in contact with blood during their clinical use, the need for hemocompatibility testing should be considered, especially when developing new materials. Development of new materials for cardiovascular applications may benefit from a more detailed hemocompatibility assessment, which could include microcirculation, cell adhesion, and leukocyte-endothelial interactions.

5.2.4 The predictability of testing for a certain material, including its debris, may benefit from the choice of study endpoints and testing approaches that incorporates clinical experience from known therapeutic applications and safety issues of similar materials.

5.2.4.1 In general, the study endpoints should be selected per their ability to measure immunomodulatory, pro/anti-inflammogenic, and tissue remodeling effects. As the examples of more specific choices, testing for an orthopedic material should take into consideration potential tissue changes such as periprosthetic osteolysis and pseudotumors, while testing for a cardiovascular material should take into consideration potential hemolytic, thrombolytic/thrombogenic, and pro-angiogenic effects.

5.2.4.2 Some endpoints currently used in effectiveness assessments can be applied to the safety assessment of adverse tissue remodeling (examples of osteogenesis-related study endpoints can be found in X1.12).

5.2.4.3 While not all possible clinical complications can be accurately replicated in animal testing models, the properly selected study endpoints for *in vivo* and supplementary *in vitro* testing can enhance the overall predictability of biocompatibility testing (more details on the choice of measurable study endpoints are provided in X1.5).

5.2.5 Rodents and other small animals (for example, rabbit, guinea pig) are traditionally used for *in vivo* biocompatibility testing models. Use of larger animal models is usually limited due to ethical and other concerns and may be reserved for models in higher need for imitating similarities with humans (weight, bone and joint structure, etc.).

5.3 *Abbreviations Used:*

5.3.1 *ALVAL*—Aseptic lymphocyte-dominated vasculitis-associated lesion.

5.3.2 *CD*—Cluster differentiation.

5.3.3 *DAMP*—Damage-associated molecular pattern.

5.3.4 *EDS/EDAX*—Energy dispersive X-ray spectroscopy.

5.3.5 *ELISA*—Enzyme-linked immunosorbent assay.

5.3.6 *FBGC*—Foreign body giant cell.

5.3.7 *FBR*—Foreign body response.

5.3.8 *FTIR*—Fourier-transform infrared (spectroscopy).

5.3.9 *H&E*—Hematoxylin and eosin.

5.3.10 *HMGB1*—High-mobility group box 1.

5.3.11 *HSP*—Heat shock protein.

5.3.12 *ICAM1*—Intercellular adhesion molecule-1.

5.3.13 *ICP-MS*—Inductively coupled plasma–mass spectrometry.

5.3.14 *Ig*—Immunoglobulin.

5.3.15 *IL*—Interleukin.

5.3.16 *LAL*—Limulus amebocyte lysate.

5.3.17 *LPS*—Lipopolysaccharide (endotoxin).

5.3.18 *MMP*—Matrix metalloproteinase.

5.3.19 *NO*—Nitric oxide.

5.3.20 *NOS/iNOS*—Nitric oxide synthase / Inducible nitic oxide synthase.

5.3.21 *PCR*—Polymerase chain reaction.

5.3.22 *ROS*—Reactive oxygen species.

5.3.23 *SAA*—Serum amyloid A.

5.3.24 *SEM*—Scanning electron microscopy.

5.3.25 *α-SMA*—Alpha-smooth muscle actin.

5.3.26 *TBARS*—Thiobarbituric acid reactive substances.

5.3.27 *TGF-β*—Transforming growth factor-beta.

5.3.28 *TLR*—Toll-like receptor.

5.3.29 *TNF-α*—Tumor necrosis factor-alpha.

5.3.30 *TRAP*—Tartrate-resistant acid phosphatase.

5.3.31 *VEGF*—Vascular endothelial growth factor.

6. Characterization Using *in Vivo* Systems

6.1 *Test and Control Material*—Characterize the nature and the range of the particles and other possible debris or degradation products (for example, ions) used, including but not limited to the following.

6.1.1 Possible sources of test and control material:

6.1.1.1 The method used to produce the test and control material for subsequent *in vivo* biological evaluation shall generate the range of particles (for example, particle size and distribution, shape, and amount or dose) and other debris (for example, generated by instruments used to surgically implant the product) or degradation products (for example, corrosion, breakdown products) reasonably expected from clinical use. The method of generation shall be described and justified. Particles or degradation products can be generated in bench testing (for example, using joint simulator machines or mechanical fatigue/corrosion testing fixtures) but may also be produced by other validated techniques, taking into account the proposed intended use. General recommendations for isolation and characterization of wear particles from bench testing are included in Practice F561 and ISO 17853. Additionally, standards are available that provide recommended loading and displacement parameters for wear testing for specific anatomical locations to generate corresponding wear particles (for example, ISO 14242-1 and 14242-3 for total hip replacement devices; ISO 14243-1 and ISO 14243-3 for total knee replacement devices; ISO 22622 for total ankle replacement devices). Device-related particles and other debris or degradation products may also be generated *in vivo* from a test material in clinical use or animal studies which replicate the conditions of a relevant end-use application. Once particles are isolated and characterized using techniques such as in Practice F561 and ISO 17853, they should be processed to remove or reduce (that is, to a biologically insignificant level) any contaminants (for example, endotoxin, chemicals used in the isolation or characterization process) and then sterilized as needed (see 6.1.2.5) to allow them to be used in subsequent biological testing.

6.1.1.2 Test articles can be prepared according to Practice F619.

6.1.1.3 Purchased or generated controls (including reference materials) should consist of particles with characteristics (for example, chemical composition, charge, size, shape, and dose) that correspond to the test particles and thus could aid in achieving their comprehensive evaluation. In addition to selection per physical/chemical characteristics with regard to the test samples, controls should be selected, when possible, per expected biological effects of a test material; the choice and appropriateness of selected control(s) should be explained. The selected controls should correspond to the range of test material related debris/degradation products. Specifically, controls should include both phagocytosable and non-phagocytosable types (for example, per size: <10 µm and ≥10 µm, respectively) that were found within the range of generated test particles, to enable comparative analysis of the test particle effects in terms of their phagocytozability.

6.1.2 The following attributes and corresponding methods should be reported for test and control materials:

6.1.2.1 Chemistry (for example, bulk material chemical composition, additives, impurities, chemical structure such as crystallinity, surface properties such as protein corona).

6.1.2.2 Size (mean and other population characteristics).

6.1.2.3 Shape (per Practice F1877).

6.1.2.4 Surface charge (if applicable).

6.1.2.5 Method of sterilization. In the event or when it is anticipated that the method of sterilization will confound medical device debris and/or degradation product generation and/or release during test, it shall be accounted and considered. If the presence of bacterial lipopolysaccharide (LPS) was quantified on test or reference materials after sterilization, specify the sensitivity of the LPS detection method.

6.1.2.6 Concentration of test material as weight, or number (of particles), or surface area/device or volumetric dose.

6.2 *In Vivo Testing*—Biological responses from the animals exposed to the medical device debris/degradation products under test should be evaluated in comparison to those derived from controls. The following controls should be considered to assist with data analysis of medical device debris/degradation products under test: negative and positive controls (for example, reference products) as well as a sham procedure control with no exposure to any test or reference material.

6.2.1 *In Vivo Models*—One or more of these models with different routes of exposure to the wear debris/degradation products can be used if appropriately qualified (see 6.4):

6.2.1.1 *Air Pouch Model*—This intradermal model has an established utility for simulating synovium and identifying particle-related immune responses. This model may be adapted for testing of devices/materials that come into contact with tissues other than synovium, but its relevance to other *in vivo* systems should be validated. The volume of air and the time allowed before introduction of the particles into the created pouches, the time points for sample collection, and the observed effects should be specified. This model is more appropriate for the assessment of acute immune responses; its appropriateness for the assessment of prolonged immune and inflammatory responses needs to be validated for the length of time of implantation. The assessment of immune responses in this model is based on evaluation of the exudates collected from the pouches. In addition to conventional testing for cell death/viability, this model allows a detailed assessment of the induced leukocytosis, for example, percentages of lymphocytes, monocytes, neutrophils, and macrophages, corroborated with the use of flow cytometry and cell-specific markers. Evaluation of the debris/degradation product induced leukocyte infiltration may be enhanced by measuring additional immune responses such as the induction of cytokines (for example, tumor necrosis factor alpha (TNFα)) or matrix metalloproteinases (for example, MMP9). The enhanced air pouch model may constitute a surrogate test for histopathology analysis and (semi)quantitative assessment of immune reactions and tissue inflammation. In addition to its use for particle debris, this model may be used to test the effects of test materials that are not particles, including gaseous device/material related products. As an additional criterion of its

suitability as a standard model, the air pouch model can be executed with limited costs and basic personnel and infrastructure.

6.2.1.2 *Cages*—Cages made of porous materials such as stainless steel mesh or porous polytetrafluoroethylene (PTFE) can be implanted subcutaneously or intraperitoneally with a test material inside the cage. The cage material and the implant location chosen should be specified and explained. The fluid accumulating in the cage should be sampled at various time intervals which should also be specified and explained. The cage and contained material removed at the termination of the experiment should be evaluated for cell adhesion, cell types, and other study endpoints including soluble products (the time chosen for termination and the choice of subsequent measurements should be explained per specifics of a test material). Fluid containing a large number of red blood cells should be discarded as representing blood and not cage fluid. As further limitations of this model, encapsulation of the cage may impact the fluid collection and corresponding cell and protein responses; more importantly, some of the observed effects may be due to the cage itself and not a test or control material.

6.2.1.3 *Bone Implant Chamber*—This is a modification of the cage system which allows determination of the effect of particles and the resulting biological response on bone remodeling and therefore is more appropriate for testing of devices/materials with orthopedic applications.

6.2.1.4 *Direct Implantation/Injection*—Direct applications of a test material via implantation (or injection if relevant to device use) are practically devoid of the delivery system related effects that may complicate interpretation of the test results from cage/chamber-based models. Intraperitoneal, intravenous, intramuscular, and subcutaneous are the favored routes in injection models; intra-articular injection is usually reserved for materials with orthopedic end applications. In general, the end use application should govern the route of injection/implantation as well as the choice and scope of organs and tissues utilized for testing.

NOTE 1—Careful dose selection and monitoring is essential to ensure animal welfare and minimization of adverse outcomes.

6.2.1.5 At the termination of the study, all sites used in these *in vivo* studies should be carefully evaluated for infection, since the presence of infection may have a major impact on the testing outcome by simulating and mimicking many test material-related inflammatory responses (more details are provided in 7.2.1). In many cases, evaluation for possible infection signs could be limited to macroscopic assessment of the test sites; in some questionable cases, additional assessments (for example, microscopic evaluation, blood/tissue culture) may be used as needed.

6.2.2 *Other Methods*—The use of other biological systems, animal models, or methods of implantation may be appropriate depending upon the intended use of the material.

6.2.3 Characterization of device-related debris/degradation products retrieved from *in vivo* studies can be performed using Practice F1877. Sample collection and processing methods applicable to testing of the tissue and biofluid specimens retrieved from both clinical and animal studies can be found in Practice F561. The specimens from test animals should be evaluated in comparison with those from control animals. See also X1.5 in this standard for additional considerations regarding the characterization of device-related debris/degradation products.

6.3 *Control Animals*—In the conduct of testing with any of the above described models, appropriate control animals who receive any vehicles, carriers, or other treatments received by the experimental models, to control for the effects of factors other than the presence of the particles, should be included as well.

6.4 *Method Qualification:*

6.4.1 For any of the methods described above, the following should be developed to support qualification for use with medical device debris/degradation products:

6.4.1.1 Provide detailed protocols and discuss any optimizations needed as compared to published methods. Describe the applicability of the method for the device-specific end use (for example, treatment period optimization, size and amount/dose of particles, debris materials that may be incompatible with the test system).

6.4.1.2 Specify and justify with supporting data, including the criteria to be used to interpret test results as compared to controls.

7. Testing Approaches, Data Analysis, and Reporting—Biological Responses

7.1 For all test and control samples, the following should be considered for analysis and reporting:

7.2 *Cellular/Tissue Response*—Cell accumulation at the site of the particles should be evaluated for relative numbers and types of cells. Standard paraffin or plastic embedded sections are usually sufficient to identify neutrophils, lymphocytes, macrophages, foreign body giant cells (FBGC), osteoclasts, osteoblasts, osteocytes, eosinophils, etc. Traditional Hematoxylin and Eosin (H&E) staining can be used in most biocompatibility-related histology and cytology applications. In some cases, special histological procedures, or immunohistochemical stains such as those described in Practice F561, or flow cytometry may be needed, for example, to confirm the identity of lymphocytes and macrophages as the main cell types involved in adaptive and innate immune responses, respectively. Overall cellular response should be characterized as focal or diffuse; its extent can be further evaluated, for example, using a scale of 0 to 5 with 0 being no cell response, 1 being accumulation of a few cells, 2 being a mild response with some cell accumulation, 3 being a moderate response, 4 being a large response, and 5 being a severe response with extensive immune/inflammatory cell accumulation. Further guidance on histopathological evaluation with (semi)quantitative scoring of cellular responses can also be found in other standards such as ISO 10993-6).

7.2.1 It should also be noted whether the response includes signs of infection that may mask or mimic inflammatory responses due to a test material. Specifically, the presence of neutrophils should be interpreted with caution. In early stages, neutrophil accumulation can indicate an acute sterile (aseptic) inflammatory response to the wear debris/degradation products

and does not necessarily indicate the presence of microbial contamination. However, if the accumulation of neutrophils persists after a few days or is discovered at later stages, it should be treated as a possible sign of infection warranting further evaluation. In some cases, termination of the study may be needed.

7.2.2 Histopathological assessment of the extent of leukocyte accumulation around the sites of initial application as well as around the sites where the particles have migrated is of an utmost importance for assessing the potential for protracted FBR with excessive inflammation and post-inflammatory tissue remodeling. The abundance of macrophages as well as the presence of multinucleated FBGCs (which represent fused macrophages) and granuloma formation should be assessed (semi-quantitatively or potentially quantitatively) as signs of FBR.

7.2.3 Using separate cell counts for lymphocytes and macrophages, the cellular/tissue responses should be considered for further characterization as predominantly innate, predominantly adaptive, or mixed (for further details on the need for inflammatory cell counting, see 5.2.2). Tissue necrosis and the extent of macrophage infiltrate (including formation of granuloma) should be considered indicators of the innate response. Perivascular lymphocyte infiltration (referred to in human orthopedic retrievals as aseptic lymphocyte-dominated vasculitis-associated lesion (ALVAL)) should be considered an indicator of the adaptive response. The combination of innate and adaptive responses should be referred to as a mixed response.

7.2.4 Phagocyte-mediated internalization and subsequent processing of wear debris can determine the degree and extent of debris-related pro-inflammatory and tissue-damaging effects. Therefore, macrophage-focused test approaches to evaluate the amount and effects of debris/degradation product uptake should be used, with quantification of the results when possible. For example, these tests can include assessment and quantification of the viability of macrophages that contain internalized particles (live versus dead/dying by apoptosis or necrosis). In addition, excessive non-internalized (extracellular) debris can be assessed, and other signs of "frustrated" phagocytosis can be evaluated. When ion release from metal-containing debris/degradation products is applicable, comparative analysis of metal ion levels from serum/blood versus those from tissues surrounding debris application and migration sites should be considered. Phagocyte-related phagolysosomal degradation products, which may be released by macrophages due to their processing of the test material and which may subsequently act as DAMPs, should be considered as measurable study endpoints for the assessment of debris-related tissue damaging potential (for more details on DAMPs, see X1.10.

7.2.5 Biodistribution and transport of particles to relevant draining organs and histologic responses in these organs should be assessed, especially when an injection model is used. Corresponding tissues from control animals should also be evaluated. The most relevant organs usually are the lymphatic draining field, spleen, liver, and kidney. In some cases, the lung may also be an appropriate draining organ when it is reasonable to suspect that particles could enter the venous return portion of the vascular system. Intravital microscopy or other imaging methods should be considered for analyzing *in vivo* biodistribution of particles and identifying their accumulation hot-spots in the living organism. Bioclearance of device-related wear debris/degradation products can be assessed by using metabolic animal cages to collect urine and fecal specimens for analysis of their renal and intestinal elimination, respectively. Biodistribution and accumulation of particles can be assessed using tissue/biofluid specimens from animals necropsied at different exposure time points. The draining lymph nodes should be harvested and assessed whenever possible. It should be noted that lymph nodes and lung commonly contain foreign material that may be confused with test or control materials due to certain characteristics (for example, birefringence) and therefore may require further evaluation. Light microscopy (with and without polarized light) of biopsied or necropsied samples from different organs/tissues is conventionally used for the assessment of biodistribution and accumulation of device-related wear debris/degradation products. Other methods such as energy dispersive X-ray analysis (EDS or EDAX), in conjunction with scanning electron microscopy (SEM), should be considered to confirm the elemental composition and other particle characteristics as necessary. For identification of other types of device-related wear debris/degradation products (for example, ions), additional methods such as Fourier-transform infrared (FTIR) spectroscopy and inductively coupled plasma–mass spectrometry (ICP-MS) should be considered if necessary.

7.3 *Measurable Cell Products and Markers*—Various tissue/biofluid specimens collected from both live and necropsied animals after exposure to a test article represent valuable sources for measuring different cellular products (markers) produced by the host in response to device-related wear debris/degradation products. The expression of cluster of differentiation (CD) markers as indicators of activated leukocyte subsets as well as the release of immunoglobulins (Ig), interleukins (IL), cell adhesion molecules, cytokines/chemokines, reactive oxygen species (ROS), and nitric oxide (NO) may be able to provide measurable means for the assessment of wear debris/degradation product-related host responses.

7.3.1 The study endpoints and methods selected to assess cellular and tissue responses should be specified and the validity of those choices should be explained with regard to the test article. Selected markers should address both the innate response and adaptive response, mainly mediated by macrophages and lymphocytes, respectively. Selected markers should also be appropriate for characterizing the host responses with regards to inflammation and post-inflammatory tissue changes (see also X1.9 – X1.13). Overall, the choice of study endpoints and methods should be sufficient for the assessment and categorization of host responses as predominantly pro- or anti-inflammatory, adaptive or innate, pro-necrotic or pro-fibrotic. Additional categorizations of wear debris/degradation product related immune and inflammatory responses and corresponding tissue changes may also be helpful.

7.3.2 Detection of the tissue-destructive potential should start by histopathological assessment of necropsied tissues for

necrosis. Supplementary *ex vivo* analysis of biopsied/necropsied tissues should be used for distinguishing between cell death types with and without pro-inflammatory response (for example, apoptosis and necrosis, respectively).

7.3.3 Selection of the study endpoints for tissue-specific remodeling effects should be aimed to address known adverse outcomes of known materials per end applications (for example, osteolysis, heterotopic ossification and/or pseudotumor for orthopedic devices; clotting, bleeding, and/or embolization for cardio/endovascular devices) and should be based on the use of cell/tissue specific specimens and markers.

7.3.4 Further details regarding the choice of study endpoints and methods which can address different debris-associated biological responses and can be further modified per the needs of a device/material under test are provided in Appendix X1.

7.3.5 In addition to the principles described above, the existing knowledge on biological effects of wear debris/degradation products from devices/materials similar to the test article can be considered. Decision-making should include consideration that novel materials or novel end applications of known materials usually require a wider scope of testing with a more comprehensive spectrum of methods and markers.

NOTE 2—The identification and study of reactive cellular products is a rapidly expanding field and any listing of specific products from which to choose would necessarily become obsolete quickly. An immunologist should be consulted to assist in the selection of substances for which testing should be performed.

8. Keywords

8.1 adaptive immune response; apoptosis; biocompatibility; biological response; cell death; chemokines; cytokines; damage-associated molecular patterns; device-related inflammogenicity and tissue remodeling potential; device-related wear debris/degradation products; *ex vivo* testing; foreign body response; inflammation; innate immune response; interleukins; internalization of debris particles by phagocytes; *in vivo* testing models; leukocyte markers; lymphocytes; macrophages; necrosis

APPENDIX

(Nonmandatory Information)

X1. RATIONALE

X1.1 In addition to the primary purpose of describing current testing approaches and the underlying biocompatibility principles based on the currently accumulated biomedical knowledge, this guide is aimed to indicate the need for and promote development of new research-based methodologies for *in vivo* testing to determine the potential biological and immunological responses to medical device-related debris and degradation products.

X1.2 It is well recognized that the biological responses to particles and other wear debris/degradation product types could be different from those to solid materials. The interaction of the particles with cells in the surrounding tissues, most notably macrophages and other phagocytic cells responsible for the uptake of particles and other debris, is one of the first steps initiating a plethora of host responses and a key to estimating the overall biological response and resultant clinical outcome.

X1.3 The interaction of foreign particles with the host tissues has been an active research area for many years. Many investigators have developed procedures for assessing these interactions, with some of these studies initially aimed at research goals other than biocompatibility, for example, nanoparticle-based theranostic and diagnostic applications. However, many of the developed testing approaches and techniques (for example, intravital imaging for evaluating biodistribution and clearance of particles) can be adapted for biocompatibility research and development of test methods for device-related wear debris and degradation products. This guide is intended to delineate the information necessary for interpretation of the results from various methodologies, regardless of whether they were initially intended or adapted for device-related biocompatibility testing.

X1.4 The interaction of device-related wear debris with the biological system is expected to result in immune cell accumulation and subsequent release of cell-cell interaction mediators that influence further progression of immune (innate and adaptive) responses and thus determine the overall biological response. Studies such as the ones described here are needed to determine the clinical relevance of these responses and to enhance the existing biocompatibility testing of devices and materials. Updated approaches from human retrieval based histopathological/histochemical analysis of peri-implant tissue responses are particularly needed in order to enhance the assessment of device wear debris/degradation product related tissue responses in animal models.

X1.5 Further research with more detailed characterization of device-related wear debris and degradation products as well as corresponding host responses is needed to develop more predictive biocompatibility testing. For instance, research on particle-protein interactions (for example, protein coronas) may result in more predictive particle characterization with regards to their expected host responses in different microenvironments. Use of ‘omics-based platforms and microfluidic (organ-on-a-chip) systems can further facilitate identification of new study endpoints and development of new techniques for complementing animal models. Where these new study endpoints and new techniques result in equivalent or better information than current animal studies, it may be possible to minimize the use of animals, thus promoting more effective and less burdensome biocompatibility testing.