Designation: F 1904 – 98 (Reapproved 2003)

Standard Practice for Testing the Biological Responses to Particles *in vivo*[1]

This standard is issued under the fixed designation F 1904; 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 the production of wear debris and degradation products from implanted materials that may lead to a cascade of biological responses resulting in damage to adjacent and remote tissues. In order to ascertain the role of particles in stimulating such responses, the nature of the responses, and the consequences of the responses, established protocols are needed. This is an emerging, rapidly developing area and the information gained from standard protocols is necessary to interpret responses. Some of the procedures listed here may, on further testing, not prove to be predictive of clinical responses to particulate debris. However, only the use of standard protocols will establish which are useful techniques. Since there are many possible and established ways of determining responses, a single standard protocol is not stated. However, this recommended practice indicates which necessary information should be supplied with test results. For laboratories without established protocols, recommendations are given and indicated with an *.

1.2 *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]

F 561 Practice for Analysis of Retrieved Metallic Orthopaedic Implants

F 619 Practice for Extraction of Medical Plastics

F 748 Practice for Selecting Generic Biological Test Methods for Materials and Devices

F 1877 Practice for Characterization of Particles

3. Summary of Practice

3.1 Biological responses to particles testing may be done using specimens from animals being tested according to the Practice F 748 matrix for irritation and sensitivity, or for implantation. Blood, organs, or tissues from the animals may be used. Procedures according to F 561 may be used to assess the cellular response.

3.2 Biological responses to particles may be tested using materials or extracts according to Practice F 619. These materials or extracts may be used in *in vivo* tests or for the *in vitro* tests. Particles generated by other methods may also be used. The method of generation must be described.

4. Significance and Use

4.1 This practice is to be used to help assess the biocompatibility of materials used in medical devices. It is designed to test the effect of particles from the materials on the host tissues.

4.2 The appropriateness of the methods should be carefully considered by the user since not all materials or applications need be tested by this practice. The validity of these studies in predicting the human response is not known at this time and studies such as described here are needed.

4.3 *Abbreviation Used*:

4.3.1 *LPS*—Lipopolysaccharide (endotoxin).

4.3.2 *LAL*—Limulus amebocyte lysate.

4.3.3 *PCR*—Polymerase chain reaction.

4.3.4 *CD*—Cluster differentiation.

4.3.5 *HLA*—Human leukocyte antigens.

5. Responses from In Vivo Systems

5.1 *Particles*—Define the nature of the particles used:

5.1.1 Source,

5.1.2 Chemistry,

5.1.3 Size (mean and range),

5.1.4 Shape,

5.1.5 Surface charge (if known),

5.1.6 Method of sterilization,

5.1.7 If the presence of bacterial lipopolysaccharide (LPS) was determined, specify how this was done and the sensitivity of the method. (LAL testing with a sensitivity of at least 0.06 EU is recommended),

[1] This practice is under the jurisdiction of ASTM Committee F04 on Medical and Surgical 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.

5.1.8 Concentration of particles used as weight, or number, or surface area/implant, and

5.1.9 Polystyrene particles, spherical, 1 to 5 μm in size should be used as a reference particle.

5.2 *Biological System*—One or more of these sites should be used:

5.2.1 *Air Pouch Model*— This is an emerging model to simulate synovial tissue. The volume of air and the time allowed before introduction of the particles should be specified. This model needs to be validated for length of time of implantation and relevance to other *in vivo* systems.

5.2.2 *Cages*—Cages made of porous materials such as stainless steel mesh or porous teflon can be implanted with a test material inside the cage. These may be implanted subcutaneously or intraperitoneally. The material and the implant location chosen should be specified. The fluid accumulating in the cage can be sampled at various time intervals. The time intervals must be specified. The cage and contained material is removed at the termination of the experiment (specify time chosen) and evaluated for cell adhesion, cell type, and products. Fluid containing large number of red blood cells should be discarded since it represents blood, not cage fluid.

5.2.3 *Bone Implant Chamber*—This is a modification of the cage system and allows determination of the effect of particles and the resulting biological response on bone remodeling

5.2.4 *Direct Injection*— Intraperitoneal, intravenous, intramuscular, subcutaneous are the favored routes. The end use application should govern the route of injection and the organ or tissue utilized in this test. Inhalation may be suitable for some end use applications.

5.2.5 Examination of tissue at implant retrieval from animal models or clinical conditions is dealt with in Practice F 1877. Some of the procedures defined here are also applicable to these tissues.

5.2.6 All sites used in these studies should be carefully evaluated for infection at the termination of the study. The presence of infection will have a major impact on the outcome since it stimulates many responses.

5.3 *Biological Response Determined*—One or more of the following:

5.3.1 Cell accumulation at the site of the particles should be evaluated for relative number and type of cells. Standard paraffin or plastic embedded sections are usually sufficient to identify acute inflammatory cells, lymphocytes, macrophages, foreign body giant cells, osteoclasts, osteoblasts, osteocytes, eosinophils, etc. But in some cases special histological procedures, or immunohistochemical stains such as those described in Practice F 561, or flow cytometry may be needed to confirm the identity of lymphocytes and macrophages. An evaluation 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 is recommended. It should also be noted whether the response is focal or diffuse.

5.3.1.1 Transport of particles to relevant draining organs and histologic responses in these organs should be determined, especially when direct injection is used. The relevant organs would be spleen, liver, kidney, and some cases the lung. The draining nodes should be harvested if identifiable. Some types of debris are distinctive (for example, carbon fibers), but lymph nodes and lung commonly contain particles and bits of birefringent stuff that may be confused with particles used in the experiment. Light microscopy with and without polarized light can be suggestive of particle migration, but other methods, (for example, EDAX, may be necessary to confirm the composition of the migrating particles. Organs from control animals should also be evaluated.

5.3.2 *Soluble Cell Products Elaborated*—This is a rapidly emerging area of technology. Histochemical and immunohistochemical techniques can be used to great advantage in these studies. Reliable reagents, kits, or hybridization protocols are available to detect the following products IL- 1β, IL-2, IL-4, IL-6, IL-10, PGE2, TNFα, immunoglobulins, as well as the lymphocyte CD markers and some HLA markers. It is not necessary to measure all of these and the selection should be based on whether there is emphasis on the macrophage response or the specific immune response.

5.3.3 Where other products from the cellular response are being detected, they should be specified and the method used specified.

5.4 Effects of the particles on other systems such as bone remodeling, chondrocyte function, cartilage repair, and synovial tissue function and repair are also important studies. The methods used should be fully described.

6. Report Section and Data Analysis

6.1 The histologic response should be compared to that of normal tissues with no particles and to that of tissues receiving the polystyrene reference particle. This may be done by counting, by digitization, by cell analyzer, or by estimation in the field of view. In some circumstances presence or absence of marker or response will suffice. In some circumstances quantitation of the response may be obtained with data on responses such as Ca++ released, enyzyme levels. DNA or RNA levels, etc.

7. Keywords

7.1 biocompatibility; biological response; *in vivo*; interleukins; particles

APPENDIX

(Nonmandatory Information)

X1. RATIONALE

X1.1 The primary purpose of this practice is to describe methodologies to determine the biological response to particles using *in vivo* responses.

X1.2 It is well recognized that the biological responses to particles could be different from those to solid materials. The interaction of the particles with cells in the tissue, notably macrophages and other phagocytic cells, is a key to the final biological response.

X1.3 The interaction of particles with host tissue has been an active research area for many years. Many investigators have developed procedures for doing these studies. This practice is intended to delineate the information necessary for interpretation of the results from these various studies and to describe methodology appropriate for those investigators developing such studies.

X1.4 The interaction of the biological system with particles will lead to the accumulation of various cells which may produce soluble mediators which influence the progression of the biological response and the immune response. It is unknown at this time which of these responses are favorable and which are unfavorable to the host. Studies such as the ones described here are needed to determine the importance of these responses in biocompatibility and biocompatibility testing of materials.