Designation: ~~F981 – 04 (Reapproved 2016)~~ F981 – 23

Standard Practice for Assessment of ~~Compatibility of Biomaterials for Surgical Implants with Respect to Effect of Materials on Muscle and Insertion into Bone~~Muscle and Bone Tissue Responses to Long-Term Implantable Materials Used in Medical Devices[1]

This standard is issued under the fixed designation F981; 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 provides ~~a series of experimental protocols for biological assays of tissue reaction to nonabsorbable biomaterials for surgical~~ guidelines for biological assessment of tissue responses to nonabsorbable for medical device implants. It assesses the effects of the material ~~on animal tissue in which it is implanted.~~ that is implanted intramuscularly or intraosseously. The experimental protocol is not designed to provide a comprehensive assessment of the systemic toxicity, immune response, carcinogenicity, ~~teratogenicity,~~ or mutagenicity of the material since other standards ~~deal with~~ address these issues. It applies only to materials with projected applications in humans where the materials will reside in bone or ~~soft~~ skeletal muscle tissue in excess of 30 ~~days and will remain unabsorbed. It is recommended that short-term assays, according to Practice~~ days. ~~F763, first be performed.~~ Applications in other organ systems or tissues may be inappropriate and are therefore excluded. Control materials ~~will consist of~~ are well recognized with a well-characterized long-term response and can include metals and any one of the metal alloys in ~~Specifications~~Specification F67, F75, F90, F136, F138, or F562, high purity dense aluminum oxide as described in Specification F603, ultra high molecular weight polyethylene as stated in Specification F648, or USP polyethylene negative control.

~~1.2 This practice is a combination of Practice F361 and Practice F469. The purpose, basic procedure, and method of evaluation of each type of material are similar; therefore, they have been combined.~~

1.2 The values stated in SI ~~units~~ units, including units officially accepted for use with SI, are to be regarded as standard. No other ~~units~~systems of measurement are included in this standard.

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

1.4 *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.*

[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 F04.16 on Biocompatibility Test Methods.

Current edition approved ~~April 1, 2016~~Sept. 1, 2023. Published ~~June 2016~~September 2023. Originally approved in 1986. Last previous edition approved in ~~2010~~2016 as F981 – 04 (2016).~~(2010).~~ DOI: ~~10.1520/F0981-04R16.~~10.1520/F0981-23.

2. Referenced Documents

2.1 *ASTM Standards:*[2]

F67 Specification for Unalloyed Titanium, for Surgical Implant Applications (UNS R50250, UNS R50400, UNS R50550, UNS R50700)

F75 Specification for Cobalt-28 Chromium-6 Molybdenum Alloy Castings and Casting Alloy for Surgical Implants (UNS R30075)

F86 Practice for Surface Preparation and Marking of Metallic Surgical Implants

F90 Specification for Wrought Cobalt-20Chromium-15Tungsten-10Nickel Alloy for Surgical Implant Applications (UNS R30605)

F136 Specification for Wrought Titanium-6Aluminum-4Vanadium ELI (Extra Low Interstitial) Alloy for Surgical Implant Applications (UNS R56401)

F138 Specification for Wrought 18Chromium-14Nickel-2.5Molybdenum Stainless Steel Bar and Wire for Surgical Implants (UNS S31673)

~~F361 Practice for Assessment of Compatibility of Metallic Materials for Surgical Implants with Respect to Effect of Materials on Tissue (Withdrawn 1987)[3]~~

~~F469 Practice for Assessment of Compatibility of Nonporous Polymeric Materials for Surgical Implants with Regard to Effect of Materials on Tissue (Withdrawn 1986)[3]~~

F562 Specification for Wrought 35Cobalt-35Nickel-20Chromium-10Molybdenum Alloy for Surgical Implant Applications (UNS R30035)

F603 Specification for High-Purity Dense Aluminum Oxide for Medical Application

F648 Specification for Ultra-High-Molecular-Weight Polyethylene Powder and Fabricated Form for Surgical Implants

F763 Practice for Short-Term Intramuscular Screening of Implantable Medical Device Materials

2.2 *ISO Standard:*[3]

ISO 10993-6 Biological Evaluation of Medical Devices—Part 6: Tests for Local Effects After Implantation

3. Summary of Practice

3.1 This practice describes the preparation of implants, the number of implants and ~~test hosts, test sites, exposure schedule, implant sterilization techniques,~~ animal models, test sites, assessment time points (that is, short-term and long-term), implant cleaning and sterilization prior to implantation, implantation method, and methods of implant retrieval and tissue examination of each test ~~site.~~ site for skeletal muscle and bone implantation. Histological criteria for evaluating tissue reaction are provided.

4. Significance and Use

4.1 This practice ~~covers a test protocol for comparing the local tissue response evoked by biomaterials, from which medical implantable devices might ultimately be fabricated, with the local tissue response elicited by control materials currently accepted for the fabrication of surgical devices. The materials may include metals (and metal alloys), dense aluminum oxide, and polyethylene that are standardized on the basis of acceptable, well recognized, long-term response.~~ is a guideline for short-term and long-term assessment of skeletal muscle and bone tissue responses to long-term implant materials. For testing of final finished medical devices, the test article for implantation shall be as for intended use, including packaging and sterilization. The tissue responses to the test article are compared to the skeletal muscle and/or bone tissue response(s) elicited by control materials. The controls consistently ~~produce~~ demonstrate known cellular reaction and wound ~~healing to a degree that has been found to be acceptable to the host.~~healing.

5. ~~Test Hosts~~Animal Model and Sites

5.1 ~~Rats~~ Laboratory rats, *Rattus norvegicus* (acceptable strains such as Fischer 344), laboratory rabbits, *Oryctolagus cuniculus* (strains such as New Zealand White ~~rabbits,~~(NZW)), and other small laboratory animals may be used as ~~test hosts for soft~~ animal models for skeletal muscle tissue implant response. It is suggested that the rats be age and ~~sex matched.~~ sex-matched. Rabbits or larger animals may be used as ~~test hosts for~~ animal models for both skeletal muscle and bone implants. When larger animals such as dogs, goats, or sheep are used, the decision should be based upon special considerations of the particular implant material or ~~study.~~study design (for example, duration, size of the implant).

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

5.1.1 All animal studies shall be done in a facility approved by a nationally recognized organization and in accordance with all appropriate regulations.

5.2 The ~~sacro-spinalis,~~sacrospinalis, paralumbar, or gluteal muscles, and the femur or tibia can serve as ~~the~~ test ~~site for implants. However, the same site must be used for test and material~~ sites for intramuscular and intraosseous implants, respectively. The implantation of the control and the test article should be performed in parallel (during the same study) with the contralateral site used for the control implants in all the ~~animal species.~~ study animals.

5.3 ~~There~~ For intraosseous implantation, there shall be a minimum of four ~~animals at each sacrifice interval~~ rabbits at each assessment time point (for example, see 6.8) for a total of sixteen rabbits per study. For implantation in the skeletal muscle, there shall be a minimum of three rabbits at each assessment time point (for example, see 6.8~~twelve animals per study.~~) for a total of twelve rabbits per study. Additional animals may be considered for longer study duration to account for potential losses in animal numbers. If larger animals are used, ~~in which a greater~~ at least two animals shall be euthanized at each time point so that an adequate number of implants ~~may be placed,~~ can be assessed (for example, see 6.5.3~~at least two animals shall be sacrificed~~), without compromising the well-being of study animals. If rats are used, at least ten rats at each time point shall be used to provide an adequate number of implants at each time ~~period.~~point. Additional animals should be considered to address systemic responses (for example, per ISO 10993-11). If assessing systemic toxicity endpoints as part of the implantation study, it is essential that separate groups of animals be used for test and control groups.

6. Implant Specimens

6.1 *Fabrication*—Each implant shall be made in a cylindrical shape with hemispherical ends (see ~~6.3~~6.4 and ~~6.4~~6.5 for sizes). If the ends are not hemispherical, this shall be ~~reported.~~ justified in the report. Each implant shall be fabricated, finished, and its surface cleaned in a manner appropriate for its projected application in human subjects in accordance with Practice F86. If the ~~specimens~~ test articles are porous, the method of preparation of the porous ~~specimens~~ test article shall be representative of the ~~contemplated human implant application~~final finished medical device and shall yield a ~~specimen~~ test article with characteristic pore size, pore volume, and pore interconnection diameter. The choice between using solid core specimens with porous coatings and specimens that are porous throughout shall be a decision of the investigator, and shall be ~~reported.~~justified in the report.

6.2 Reference metallic specimens shall be fabricated in accordance with 6.1 from materials such as the metal alloys in ~~Specifications~~Specification F67, F75, F90, F138, or F562, ceramic in Specification F603, or polymers such as in Specification F648 polyethylene or USP Negative Control Plastic. If the test materials are porous, consideration should be given to using porous ~~specimens~~articles for reference ~~specimens.~~controls. Alternatively, nonporous reference ~~specimens~~controls may be used. The choice of reference controls shall be reported and justified.

6.3 To assess the impact of surgical procedure, sham controls may be helpful. If sham controls are used, the same implantation procedure with the test or control should be used.

6.4 *Suggested Sizes and Shapes of Implants for ~~Insertion~~Implantation in Skeletal Muscle:*

6.4.1 The implants shall be cylindrical in shape and may range from 1 mm to 6 mm in diameter and from 10 mm to ~~20 mm~~ 20 mm in length depending upon the relative size of the species under study.

6.4.2 The dimensions used shall be reported in accordance with 8.1.

6.4.3 Depending upon the particular device application, other sample shapes may be used. For instance, an investigator might wish to test the biocompatibility of a new material for ~~screws in the form of a screw. If an alternative specimen~~ a medical device in its final finished form. If an article with an alternative shape is used, this should be reported and justified in accordance with 8.1.

6.5 *Sizes and Shapes of Implants for ~~Insertion in Bone:~~ Intraosseous Implantation:*

6.5.1 Implant ~~diameters for use in bone shall be approximately equal to the cortex thickness.~~ shall be cylindrical in shape and the dimension may range from 2 mm to 4 mm in diameter and from 6 mm to 12 mm in length depending upon the animal model used in the study. Implant lengths shall allow ~~them~~ the test or control article to reside in ~~one~~the cortex and the ~~medulla~~ medullary cavity without excessive protrusion beyond the ~~periosteum.~~ level of the periosteum.

6.5.2 Depending upon the particular device application, other sample shapes that are anatomically compatible may be used.

6.5.3 The dimensions and shapes used shall be reported and justified in accordance with 8.1.

6.6 *Number of Test and Control Implants:*

~~6.5.1 In each rat, due to size, there may be two implants; one test and one control material implant.~~

6.6.1 ~~In each rabbit, due to size, there may be six implants; four test and two control material implants.~~Selection of the animal model and animal numbers in the study shall depend on body weight, anatomical location for implantation, and implant characteristics (for example, size, configuration). Ten test and ten control material implants shall be assessed at each time point and shall be from at least three different animals for skeletal muscle implantation and at least four different animals for intraosseous implantation.

~~6.5.3 In larger animals, there may be twelve implants; eight test material and four control material implants.~~

~~6.5.4 In rabbits or larger animals, at least sixteen test materials and eight materials shall be tested at each time period.~~

6.7 ~~*Conditioning:*~~ *Cleaning, Sterilization, and Conditioning (per device intended use):*

6.7.1 Remove all surface contaminants with appropriate ~~solvents~~ cleaning agents and rinse all test and control implants in distilled water prior to sterilization. It is recommended that the implant materials be ~~processed and cleaned~~processed, cleaned, packaged, and sterilized in the same way the final product will be.

~~6.6.2 Clean, package, and sterilize all implants in the same way as used for human implantation.~~

6.7.2 After final preparation and sterilization, handle the test and control implants with great care to ensure that they are not scratched, damaged, or contaminated in any way prior to insertion. If there is device-specific preparation (for example, delivery through a catheter) this should be included as a conditioning step prior to implantation.

6.7.3 Report all details of cleaning, sterilization, and conditioning in accordance with 8.1.

6.8 *Implantation Period*—Insert all implants into each animal at the same surgical session for implantation periods of 4, 12, 26, and 52 weeks. Justification shall be provided for the selection of additional time points.

7. Procedure

7.1 All the procedures shall be performed using sterile techniques.

7.2 ~~*Implantation (Muscle):*~~*Intramuscular Implantation:*

~~7.1.1 Place material implants in the paravertebral muscles in such a manner that they are directly in contact with muscle tissue.~~

7.2.1 Introduce material implants in ~~larger~~ animals by ~~the technique of making an implantation site in the muscle~~ directly inserting the material intramuscularly or by using a hemostat to separate the muscle ~~fibers.~~ fibers creating an implantation site. Then insert the implant into the site using plastic-tipped forceps or any tool that is nonabrasive to avoid damage to the implant.

7.2.2 Introduce material implants using sterile technique. Sterile disposable needles or hypodermic tubing and trochar may be used to implant the material implants into the ~~paravertebral muscles along the spine. In rats, insert a~~muscles. In rats and rabbits, insert the negative control implant on one side ~~of the spine and a~~and the test material implant on the ~~other side. In rabbits, implant one negative control material on each side of the spine and implant two test materials on each side of the spine.~~ contralateral side. If larger diameter specimens are used, an alternative implantation technique is ~~that~~ described in ~~7.1.2~~7.2.1.

7.3 ~~*Implantation (Femur)—*~~*Intraosseous Implantation*—Expose the ~~lateral cortex of each rabbit femur~~ cortical surface of the bone of each rabbit or larger animal and drill undersized pilot holes through the ~~lateral~~ cortex using the technique and instrument appropriate for the procedure. Final reaming of the holes should be performed by hand using an appropriate size reamer to yield

holes which are smaller than the diameter of the implant specimens by 0.1 mm or less. Test article handling prior to and during insertion should be conducted using gloved hands or using a tool such as a forceps. However, care should be taken if using forceps as they can cause damage to the surface of the test article. Furthermore, the bones are fragile and can be easily damaged during implantation. Into each one of these holes, insert one of the implants by finger pressure. Then close the wound.

NOTE 1—Caution should be taken to minimize the motion of the implant in the tissue to prevent the effects of motion on the desired result.

7.4 *Postoperative Care:*

~~7.3.1 All animal studies shall be done in a facility approved by a nationally recognized organization and in accordance with all appropriate regulations.~~

7.4.1 Carefully observe each animal at least once daily during the ~~period of assay~~ study period and record all observations and report any abnormal findings.

7.4.2 Infection or injury of the test implant site may invalidate the results. The decision to replace the animal so that the total number of retrieved implants will be as represented in the schedule shall be dependent upon the design of the ~~study.~~study and shall be justified.

7.4.3 If an animal dies prior to the expected date of ~~sacrifice,~~euthanasia, perform a necropsy in accordance with the procedure in ~~7.4~~7.5 to determine the cause of death. Replacement of the animal to the study shall be dependent upon the design of the study. ~~Include the animal in the assay of data if the cause of death is related to the procedure or test material.~~ The study results shall be reported for all the animals implanted in the study.

7.5 *~~Sacrifice~~Euthanasia and Implant Retrieval:*

7.5.1 Euthanize animals ~~by a humane method~~ at the intervals specified in ~~6.7~~6.8.

~~NOTE 2—The necropsy periods start at 12 weeks because it is assumed that acceptable implant data has been received for earlier periods from short term implant testing according to Practice F763. If the 90-day sacrifice period has been utilized under Practice F763, that group need not be repeated under this protocol, and thus, the 12-week group may be eliminated.~~

7.5.2 ~~At necropsy, record any gross abnormalities of color or consistency observed in~~ In accordance with standard laboratory practice, perform a necropsy on all animals that are euthanized for the purposes of the study or unexpectedly died during the study period. Record all gross observations (for example, color, consistency) of the tissue surrounding the implant. Remove each implant with an intact envelope of surrounding tissue. ~~Include in the tissue sample~~ To encompass the tissue responses to the implant, it is recommended to include a minimum of a ~~4-mm~~ 4 mm thick layer of tissue surrounding the implant. ~~If less than a 4-mm thick layer of tissue is removed, report~~ Report the findings in accordance with 8.1.

~~7.5 *Postmortem Observations*—In accordance with standard laboratory practice, perform a necropsy on all animals that are sacrificed for the purposes of the assay or die during the assay period. Establish the status of the health of the experimental animal during the period of the assay. Report as described in Section 8.~~

7.6 *Histological Procedure:*

7.6.1 *Tissue Sample Preparation*—Prepare appropriate blocks from each implantation ~~site and indicate the orientation of the axis of the femur relative to the axis of the implant for bone implants. Also indicate the orientation of the implant relative to the axis of rotation of the femoral condyles.~~site.

7.6.1.1 Process the excised tissue block containing either a test implant or control implant for histopathological examination and ~~such~~ other studies, as ~~are appropriate. Cut the sample midway from end to end into appropriate size and~~ appropriate. Cut in the appropriate orientation for each study. ~~Transfer, or record, or both, the orientational details noted in 7.6.1 to each part of the sample.~~ Record the gross appearance of the implant and the tissue. If the sample is porous, it is imperative that sectioning procedures be used that maintain the implant within its tissue envelope to allow the evaluation of tissue within the pores. Such procedures may include plastic embedding and ground section preparation.