Designation: F 763 – 04

Standard Practice for Short-Term Screening of Implant Materials[1]

This standard is issued under the fixed designation F 763; 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 guidelines for short-term testing or screening of candidate materials, both porous and dense, as to the effects of the material on animal tissue in which it is implanted. This is a rapid screening prodedure for determining acceptability of candidate materials.

1.2 This practice, along with other appropriate biological tests (including other appropriate ASTM tests) may be used in the biocompatibility assessment of the candidate materials for use in the fabrication of devices for clinical application.

1.3 This experimental protocol is not designed to provide a comprehensive assessment of the systemic toxicity, carcinogenicity, teratogenicity, or mutagenicity of the material since other standards deal with these issues.

1.4 This practice is one of several developed for the assessment of the biocompatibility of materials. Practice F 748 provides guidance for the selection of appropriate methods for testing materials for a specific application.

2. Referenced Documents

2.1 *ASTM Standards:* [2]

F 75 Specification for Cobalt-28Chromium-6Molybdenum Alloy Castings and Casting Alloy for Surgical Implants (UNS R30075)

F 86 Practice for Surface Preparation and Marking of Metallic Surgical Implants

F 90 Specification for Wrought Cobalt-20Chromium-15Tungsten-10Nickel Alloy for Surgical Implant Applications (UNS R30605)

F 136 Specification for Wrought Titanium-6Aluminum-4Vanadium 4V ELI (Extra Low Interstiitial) Alloy for Surgical Implant Applications (UNS R56401)

F 138 Specification for Wrought 18Chromium-14Nickel-2.5Molybdenum Stainless Steel Bar and Wire for Surgical Implants (UNS S31673)

F 562 Specification for Wrought Cobalt-35Nickel-20Chromium–10Molybdenum Alloy for Surgical Implant Applications (UNS R30035)

F 563 Specification for Wrought Cobalt-20Nickel-20Chromium-3.5Molybdenum-3.5Tungsten-5Iron Alloy for Surgical Implant Applications (UNS R30563)

F 603 Specification for High-Purity Dense Aluminum Oxide for Surgical Implant Application

F 648 Specification for Ultra-High-Molecular-Weight Polyethlene Powder and Fabricated Form for Surgical Implants

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

F 981 Practice for Assessment of Compatibility of Biomaterials for Surgical Implants with Respect to Effect of Materials on Muscle and Bone

3. Terminology

3.1 *Description of a Term Specific to this Standard:*

3.1.1 *biocompatibility assay*—a comparison of the tissue response produced through the close association of the implanted candidate material to its implant site within the host animal to that tissue response recognized and established as suitable with control materials.

4. Summary of Practice

4.1 Under aseptic conditions, test specimens of the candidate material and of controls are inserted into a muscle or group of muscles of the animal host. After a period of time the animals are euthanized. The tissue reactions to implants of the candidate material during the acute to subchronic time period of healing are compared with tissue reactions to control materials which have a well characterized response. The implants are not subject to major stress while *in situ*.

5. Significance and Use

5.1 The use of *in vivo* implantation techniques for characterizing the biocompatibility of materials to be utilized in various medical applications provides a unique assessment of such materials not achieved by other procedures. Physical characteristics (that is, form, density, hardness, surface finish) can influence the character of the tissue response to the test materials.

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[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.

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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.

5.2 This practice is intended as a rapid screening procedure for determining the acceptability of candidate materials. It would be invoked prior to using the long-term tests described in Practice F 981. It is understood that for some applications additional tests, including long-term implantation studies, may be required to assess the final suitability of the candidate materials.

5.3 This practice may not be appropriate for all types of implant applications. The user is cautioned to consider the appropriateness of the method in view of the materials being tested, their potential applications, and the recommendations contained in Practice F 748.

6. Test Preparation

6.1 Rabbits, rats, or other animals may be used as test hosts. The following procedure is written for New Zealand white rabbits, a commonly used test host but the procedure can be adapted with few alterations to other test hosts.

6.2 *Test Hosts and Sites*:

6.2.1 Choose healthy adult rabbits that weigh more than 2.5 kg and whose paravertebral muscles are sufficiently large to allow for implantation of the test specimens.

6.2.2 The paravertebral muscle shall serve as the test site for implants. (The gluteal muscles of rats have been used as test sites by some investigators.)

6.2.3 *Preparation of Rabbits*—On the day of the implantation or up to 20 h before implantation, clip the fur of the animals on both sides of the spinal column. Remove loose hair.

6.3 *Selection of Control Materials*:

6.3.1 Selection of control material(s) should be based on their prior acceptable use in medical applications similar to those proposed for the candidate test material and is not restricted to those listed in 6.3.2.

6.3.2 Metallic control materials, which have been demonstrated to elicit minimal tissue reactions, are the metal alloys, such as in Specifications F 75, F 90, F 136, F 138, F 562, or F 563, or a ceramic, such as, alumina F 603. A suitable polymeric control material is found in polyethylene Specification F 648.

NOTE 1—There are times when use of a positive control can help to clarify the character of the tissue response to the candidate test sample.

6.3.3 If the most appropriate control material is expected to elicit a tissue response greater than that normally observed with Negative Control Plastic or the alloys cited above, samples of these latter materials may be implanted as controls on the surgical technique.

7. Test Specimens

7.1 *Fabrication*—Each implant shall be fabricated, finished, and its surface cleaned in a manner appropriate for its projected application in humans. Dense metal implants should be finished in accordance with Practice F 86. The size, shape, and surface of test and control implants shall be as similar as is practically possible.

7.2 Implant sizes are left to the discretion of the investigator. Implants in the size range 1 by 10 mm (0.04 by 0.4 in.) to 3.2 by 12 mm (0.125 by 0.5 in.) have often been used. They may be of circular or square cross section. The edges of the specimens should be as smooth as possible to avoid additional mechanical trauma upon implantation.

7.3 *Implantation Period*:

7.3.1 The insertion of all implants into any one animal shall be done at the same surgical session.

7.3.2 Implant evaluation should be performed at 7 and 30 d so that an accurate characterization of both the test and control materials can be made during the acute and subchronic stages of the healing tissue response. Three animals will be used for each sample period, that is, 3 at 7 d, and 3 at 30 d.

NOTE 2—Some investigators have found that extending the test to include a third group of animals maintained for 90 d can provide additional data on the host response to the implant material.[3]

8. Procedure

8.1 *Implantation*:

8.1.1 The recommended method of implantation is by hypodermic needle or tube and trochar. For larger diameter samples, an incision of appropriate size will be required to permit passage of the larger diameter tube. If this technique is not convenient, however, other equivalent implantation techniques judged appropriate may be used. These should be reported as in 9.1. The implantation must be done using aseptic procedures.

8.1.2 *Preparation of Test Specimens*—The specimens should be fabricated as described in 7.1 and prepared for implantation following the procedure in either 8.1.2.1 or 8.1.2.2.

8.1.2.1 Sterilize each specimen as appropriate for final application and, using aseptic technique, insert it into a sterile needle or tube; or,

8.1.2.2 Insert the specimen into a needle or tube, protect the ends with an appropriate cover, and sterilize the assemblies in an appropriate manner.

NOTE 3—Allow for proper degassing if sterilizing agents such as ethylene oxide are used.

NOTE 4—If the materials to be tested are harder than the materials from which the handling instruments are made, there is the danger of surface contamination of the test specimens by wear from the instruments which can disturb the results (for example, ceramic test specimens implanted with metal instruments). If such test specimens must be handled, soft textile or plastic should be used between the implants and the instruments. Of course, care must be taken that none of these auxiliary protecting materials remain in the implantation wound.

8.1.3 The animals should be anesthetized with a commonly used anesthetic agent to a degree deep enough to prevent muscular movement, such as twitching. Properly scrub the clipped skin surface of the animal.

8.1.4 Implant four specimens of the sample into the paravertebral muscle on one side of the spine of each rabbit, about 2.5 cm from the mid-line and parallel to the spinal columns, and about 2.5 cm apart from each other. In a similar fashion, implant four specimens of the control material in the corresponding muscle on the opposite side of the spine of each animal.

[3] Turner, E., Lawrence, W. H., and Autian, J., "Subacute Toxicity Testing of Biomaterials Using Histopathological Evaluation of Rabbit Muscle Tissue," Journal of Biomedical Materials Research, Vol 7, 1973, pp. 39–58.

8.1.5 In cases where the negative control is other than specified in 6.3.2 and may be expected to elicit more than a minimal response, use only two test specimens of this negative control. Implant these two control specimens in a location that will not interfere with the test samples.

8.1.6 When using a sterile needle for insertion, insert a sterile stylet into the needle to hold the test specimen in the tissue while withdrawing the needle. With trocar implantation, insert the test specimen after withdrawing the central point and use a stylet to hold the sample while withdrawing the cannula.

8.1.7 If excessive bleeding is observed after implantation of a test specimen, place a duplicate test specimen at another site. Close the incision after implantation is complete, if applicable. If a test host other than the rabbit is chosen, use as many animals as are necessary to permit the implantation of 12 test specimens and 12 controls for each sacrifice interval.

8.2 *Postoperative Care*:

8.2.1 All animal studies must be done, in a facility approved by a nationally-recognized organization and in accordance with all appropriate regulations.

8.2.2 Carefully observe each animal during the period of assay and report any abnormal findings.

8.2.3 If an animal dies prior to the expected date of sacrifice, perform a necropsy to determine the cause of death. Include the animal in the assay of data if the cause of death is related to the procedure or test material.

8.2.4 A successful test is one in which eight test specimens and eight controls for each test period are available for histologic evaluation.

8.2.5 Should infection or injury of the test implant site invalidate the results, replace the animal with another if necessary as described in 8.2.4.

8.3 *Sacrifice and Implant Retrieval*:

8.3.1 Sacrifice animals at the intervals suggested in 7.3.2.

8.3.2 At sacrifice, record any gross abnormalities of color or consistency observed in the tissue surrounding the implant. Documentation of findings using photography for a permanent record is highly recommended.

8.4 *Gross Observations, Acute Test (7 d) and Subchronic Test (30 d)*:

8.4.1 Macroscopically examine the area of the tissue surrounding the center portion of each implanted test specimen. Use a magnifying glass, if necessary.

8.4.2 The requirements of the test are met if, in each rabbit, the reaction to three of four sample test specimens is not significantly greater than that of the reaction to the corresponding negative control. In situations in which two types of negative controls are included, these criteria apply to the tissue surrounding specimens of the minimally reactive material.

8.5 *Histopathological Observation, Acute Test (7 d), and Subchronic Test (30 d)*:

8.5.1 *Tissue Sample Preparation*:

8.5.1.1 Remove each implant with an intact envelope of surrounding tissue. The tissue sample should include a 4-mm thick layer of tissue surrounding the implants. If less than 4 mm of tissue is removed, report in accordance with 9.1. Process the excised tissue block containing either a test implant or control implant for histopathological and such other studies as are appropriate. Cut the tissue sample into appropriate specimens for each study. Record the gross appearance of the implant and the tissue immediately adjacent to the implant as to consistency and color, as seen by the naked eye, and with a hand lens or stereomicroscope (see 9.2).

8.5.1.2 If possible, process the specimen with the implant in place. This allows easy identification of the implant site and minimizes distortion during fixation. After fixation, the implant material can be removed if necessary, and the tissue specimens processed for sectioning using standard laboratory practice for embedding and staining. If an implant is removed from its tissue bed, report the amount of tissue removed with the implant as required in 9.1.

8.5.1.3 Histological sections can be prepared using conventional microtomy section, or grinding of plastic embedded specimens as appropriate.

8.5.1.4 For porous implant materials, the quality and quantity of tissue ingrowth may also be examined using the appropriate prepared sections.

8.5.2 *Histopathological Schedule*:

8.5.2.1 Process at least two tissue microscope slides from each sample of control and test implant, including the tissue envelope surrounding the implant so as to obtain adequate histopathological assessment of tissue reaction.

8.5.2.2 If special stains are deemed necessary, prepare additional sections, and make appropriate observations.

8.5.3 *Histopathological Observations*—Compare the amount of tissue reaction adjacent to the test implant to that adjacent to a similar location on the control implant as regards thickness of scar, presence of inflammatory or other cell types not normally present at the site, presence of particles, and any other indications of an interaction between tissue and material. The use of a scoring system similar to that in Practice F 981 is highly recommended. Report in accordance with 9.3.

8.5.4 *Evaluation*—The requirements of the histopathological evaluation are met if, in each rabbit, the histopathological reaction to three of the four sample test specimens is not significantly greater than the reaction to the corresponding negative control. In situations in which two types of negative controls are included, these criteria apply to the tissue surrounding specimens of the minimally reactive material. The results of histological evaluation shall carry more weight than those of gross evaluation in determining suitability for an implant material.

9. Report

9.1 A report shall be made to include all details of implant characterization and size, fabrication, conditioning, (including cleaning, handling, and sterilization techniques employed), and procedures for implantation and implant retrieval. The control material characteristics of chemical composition, mechanical and thermal condition, and finish should be reported. Also details of any special procedure (such as unusual or unique diet fed to test animals) shall be included in the report.

9.2 A report shall be made to include the observations of each control and test implant at each time interval, as well as the gross appearance of the surrounding tissue in which the implants were implanted.

9.3 A report shall be made on the observation of each histopathological examination.

10. Keywords

10.1 biocompatibility; biomaterials; New Zealand white rabbits; orthopaedic medical devices; short-term implantation; tissue compatibility

APPENDIX

(Nonmandatory Information)

X1. RATIONALE

X1.1 Within ASTM, there is a standard which addresses the question of material biocompatibility through *in vivo* testing: Practice F 981. This practice is intended as a long-term test with experiments running as long as 2 years, therefore manufacturers, implant developers, and researchers cannot use it on a routine basis to rapidly test or screen candidate materials.

X1.2 This testing protocol, patterned after the USP-Implantation Test which calls for tests of very short duration (3 d), is intended as a short-term screening test for candidate materials. It would be invoked prior to using the long-term test of Practice F 981.

X1.3 Both porous and dense candidate materials are included within the scope of this practice and their effects on tissue are compared to dense control materials. There are, of course, very large differences between porous and dense materials; however, porous negative controls are not yet available. Hence, provision is made for comparing the tissue effects of the porous candidate material to both other suitable controls of a like material and to dense negative controls. This same problem also applies to certain polymers that have proven to have application as medical implants but which elicit a greater tissue response than the dense negative control. Such candidate materials can also be compared to other similar, already medically accepted materials as well as dense negative controls.

X1.4 This practice excludes, as beyond its scope, biodegradable implants or other biological implants such as gels, liquids, allografts or xenografts and immune responses to implanted materials.