Designation: F1983 – 23

Standard Practice for Assessment of Selected Tissue Effects of Absorbable Biomaterials for Implant Applications[1]

This standard is issued under the fixed designation F1983; 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 experimental protocols for biological assays of tissue reactions to absorbable biomaterials for implant applications. This practice applies only to absorbable materials with projected clinical applications in which the materials will reside in bone or soft tissue longer than 30 days and less than three years. Other standards with designated implantation times are available to address shorter time periods. Careful consideration should be given to the appropriateness of this practice for slowly degrading materials that will remain for longer than three years. It is anticipated that the tissue response to degrading biomaterials will be different from the response to nonabsorbable materials. In many cases, a chronic inflammatory response may be observed during the degradation phase, but the local histology should return to normal after absorption; therefore, the minimal tissue response usually equated with biocompatibility may require long implantations.

1.2 The time period for implant absorption can depend on variables of chemical composition, implant size, implant location, and animal models. Therefore, the selected time points for assessing tissue effects may be selected based on the rate of absorption.

1.3 These protocols assess the effects of the material on the animal tissue in which it is implanted. They do not fully assess systemic toxicity, carcinogenicity, reproductive and development toxicity, or mutagenicity of the material. Other standards are available to address these issues.

1.4 To maximize use of the animals in the study protocol, some aspects of systemic toxicity, including effects of degradation products on different organs and tissues downstream of or surrounding the target site, can be addressed with this practice.

1.5 Because animal models are not identical to human biology, this practice cannot account for all potential biological hazards, for example the effect of the oligosaccharide a-Gal (Gala 1,3-Galb1-4GlcNAc-R), known as the "a-Gal" epitope present in xenogeneic materials on humans. See ISO 22442.

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

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

F981 Practice for Assessment of Compatibility of Biomaterials for Surgical Implants with Respect to Effect of Materials on Muscle and Insertion into Bone

F1408 Practice for Subcutaneous Screening Test for Implant Materials

F1635 Test Method for ***in vitro*** Degradation Testing of Hydrolytically Degradable Polymer Resins and Fabricated Forms for Surgical Implants

F1903 Practice for Testing for Cellular Responses to Particles ***in vitro***

F1904 Practice for Testing the Biological Responses to Particles ***in vivo***

F2902 Guide for Assessment of Absorbable Polymeric Implants

F3268 Guide for ***in vitro*** Degradation Testing of Absorbable Metals

[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.

2.2 *ISO Standards:*[3]

ISO 10993-6 Biological evaluation of medical devices—Part 6: Tests for local effects after implantation

ISO 10993-9 Biological evaluation of medical devices—Part 9: Framework for identification and quantification of potential degradation products

ISO 10993-11 Biological evaluation of medical devices—Part 11: Tests for systemic toxicity

ISO 10993-18 Biological evaluation of medical devices—Part 18: Chemical characterization of medical device materials within a risk management process

ISO/TS 10993-19 Biological evaluation of medical devices—Part 19: Physico-chemical, morphological and topographical characterization of materials

ISO 13781 Implants for surgery—Homopolymers, copolymers and blends on poly(lactide)—In vitro degradation testing

ISO/TS 17137 Cardiovascular implants and extracorporeal systems—Cardiovascular absorbable implants

ISO 22442 Medical devices utilizing animal tissues and their derivatives

ISO/TS 37137-1 Biological evaluation of absorbable medical devices—Part 1: General requirements

3. Terminology

3.1 *Definitions:*

3.1.1 *final finished form*—a device or device component that includes all manufacturing processes for the "to be marketed" device including packaging and sterilization, if applicable.[4]

4. Summary of Practice

4.1 Under strict aseptic conditions, sterile test articles (for example, final device) are implanted into a relevant animal model and at a clinically relevant anatomical tissue site. However, for screening candidate materials, testing in a clinically relevant animal model and anatomical tissue site may not be necessary. Small laboratory animals such as mice, rats, hamsters, or rabbits are preferred. In addition, the use of larger animals, such as the dog, goat, pig, or sheep may be justified based upon special considerations of the particular study. Choice of the animal model should also consider the availability of historical data on biological responses of these animals to similar devices to aid in analysis and comparison of the data obtained.

4.2 All animal studies shall be done in a facility in accordance with all appropriate regulations.

5. Significance and Use

5.1 This practice is a guideline for a screening test of candidate materials or assessment of local tissue response to absorbable medical devices which are expected to undergo complete absorption within three years.

5.2 This practice is similar to those for studies on candidate materials or medical devices that are not absorbable, such as those specified in Practices F763, F981, and F1408; however, analysis of the host response must take into account the effect of degradation and degradation products on the inflammatory response at the local tissue site and on subsequent healing of the implantation site, as well as the potential for adverse distal tissue effects.

5.3 For testing of absorbable medical devices, the test article for implantation should be in the final finished form as for intended use, including packaging and sterilization (if applicable). Configurations specific to the animal study may be needed. The test article's surface-area-to-body mass or mass-to-body mass ratios within the animal model should be established by calculating based on surface-area-to-body mass or mass-to-body mass ratios in humans during the device's intended clinical use. Worst-case clinical dose should be considered in the study design. For implantation studies incorporating evaluation of both local tissue responses and systemic toxicity, exaggerated material surface area or mass-to-body mass ratios (for example, a 2X to 10X safety factor to assess implant safety for regulatory submissions) compared to clinical use (for example, largest device size, maximum number of devices) should be considered, unless otherwise justified. For example, implantation of exaggerated doses may not be feasible in the selected animal model. For some devices, additional animal group(s) for exaggerated conditions should be considered if dose response information is needed. Additionally, for some devices, exaggerated dose at a specific implantation site can also be used to evaluate local tissue responses.

5.4 Materials that are designed for use in devices with *in situ* polymerization shall be introduced in a manner such that *in situ* polymerization occurs. Additional testing of individual precursor components or partially polymerized materials may be needed in some cases (for example, if testing of the final implant indicates an adverse response or incomplete polymerization).

6. Animal Model

6.1 The choice of animal model shall take into consideration the normal life span of the animal, the clinical use conditions, device absorption kinetics, and the length of the implantation study, and shall be justified. The strain, sex, age, weight, origin, and general health of the animals used should be recorded. Institutional and government animal use and care policies and regulations shall be followed.[5,6,7,8,9]

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

[4] FDA Biocompatibility Guidance, "Use of International Standard ISO 10993-1, 'Biological evaluation of medical devices—Part 1: Evaluation and testing within a risk management process'" (https://www.fda.gov/media/85865/download).

[5] Animal Welfare Act, 7 U.S.C. § 2131 et seq., as amended. 2013.

[6] Animal Welfare Regulations, 9 CFR Chapter 1, Subchapter A, Parts 1, 2, and 3. 2004.

[7] Health Research Extension Act of 1985, Public Law 99-158 November 20, 1985.

[8] Office of Laboratory Animal Welfare, National Institutes of Health. Public Health Service Policy on Humane Care and Use of Laboratory Animals, Bethesda, MD, 2015.

[9] National Research Council, Guide for the Care and Use of Laboratory Animals: Eighth Edition. Washington, DC, The National Academies Press; 2011.

6.2 The number of implant sites shall depend on the size of the implant and the animal. The distance between implants shall be sufficient so that separate tissue blocks are prepared easily for each implant and that the local biological reactions do not overlap or interfere with each other. Implants may be placed bilaterally in soft tissue, including muscle. Bilateral implantation into bone should be considered carefully and justification given. In general, mice, rats, hamsters, and other small laboratory animal species should receive no more than one implant on each side. Larger animals, including rabbits, may receive up to five implants on each side. When the implant is composed of a collection of particles, pellets, and so forth, each collection is considered one implant site.

6.3 Scientifically established analytical methods should be used in the identification and quantification of degradation products (ISO 10993-9, ISO 10993-18, ISO 10993-19, ISO 13781, Test Method F1635, Guide F2902, Guide F3268). Literature information (if available) on the fate of the absorbable material's degradation products can be used to address their absorption, distribution, metabolism, and excretion (ADME) and identify the potential organs involved. Literature evaluations should focus on all degradation products, including those from major compositional components as well as any other constituents with known or suspected toxicities, at the amounts present that could impact tissue response.

NOTE 1—A pilot study *in vitro* or in small animals may be undertaken to assess the rate of degradation which can potentially be used to select estimated time points for evaluating degradation in large animal studies.

NOTE 2—In some cases where degradation products or metabolites of the candidate material are not known or well established, it may be possible to assess the quantitation and distribution of degradation products or metabolites using *in vivo* radio-labeling methods following administration of radio-labeled parent material for ADME assessments. However, if radio-labeling is used, a justification should be included to explain why radio-labeling is not expected to impact ADME results.

7. Test Article and Implant Placement Considerations

7.1 *Test Article*—May be devices in their final finished form or made from candidate materials in configurations specific for the animal study. Photograph(s) of the implant articles should be taken prior to implantation. As described in 5.3, the material/host ratio should be selected based on clinical use, with material/body mass ratios of 2X and 10X, if applicable. Relevant configurations of implant articles, such as cylinders, flat cloths, amorphous gels, and polymerizable liquids may be used for material screening studies.

7.2 The implantation site of the absorbable device or candidate material shall be described and recorded with anatomic landmarks and include adequate means to identify the specific implant sites, including during and after advanced stages of degradation. Such means of site identification may include use of an implanted non-absorbable marker or other permanent method, such as a template.

7.3 Control materials shall be implanted using the same placement techniques as the test material to allow the comparison of the tissue response. Choice of control devices/materials with established biocompatibility and clinical relevance shall adhere to the following selection priority with appropriate consideration for clinical best practice, availability, and dimensional suitability for the intended implantation site:

(1) Absorbable device/material with similar expected absorption profile.

(2) Absorbable device/material with different absorption profile or non-absorbable device/material.

NOTE 3—Absorbable device/material controls that possess a different degradation rate than the test implant may require retrieval at additional intervals to allow assessment of tissue response at an equivalent stage in the control material's degradation/absorption process. Use of an absorbable device/material with an absorption profile different from the test implant may not allow a bilateral implantation and additional animals may be used for the implantation of the control device/material.

(3) Sham sites / sham animals—A sham surgical site (to assess local effects), or a sham surgical animal (to assess local and systemic effects) may be helpful. If a sham site or sham animal is used, the same implantation procedure without the test or control should be used.

NOTE 4—Such sites may be used to assess the impact of surgical procedures but may not enable a direct comparison of tissue responses to the ongoing presence of an implant (absorbable or nonabsorbable).

7.3.1 The material/host (material surface area or mass-to-body mass) ratio of any control material should be comparable to the material/host ratio used for the test implant as described in 5.3. The selection of the control shall be justified. Guidance regarding considerations prior to commencing an *in vivo* study of absorbable materials can be found in the following standards:

(1) Guide F2902—For absorbable polymeric devices, this standard describes the manufacturing, characterization, packaging, sterilization, and biocompatibility aspects and the related testing that should be considered prior to undertaking *in vivo* evaluations.

(2) ISO 10993-6—Provides absorbable-specific considerations when evaluating a material's biological safety through implantation, which includes guidance for selecting appropriate animal retrieval intervals (see Clause 5 of ISO 10993-6:2016).

(3) ISO/TS 17137—Provides recommendations on *in vitro* and *in vivo* assessments of absorbable (test or control) implants (see the stages of degradation depicted in Figure 2 and the supporting discussions contained within subclauses 5.1, 5.3, 5.4, and 5.6 in ISO/TS 17137:2021).

(4) ISO/TS 37137-1—Provides supplemental absorbable-specific considerations when biologically evaluating a device in accordance with the ISO 10993 series.

7.3.2 If assessing systemic endpoints as part of the implantation study, it is essential that separate groups of animals be used for test and control groups.

7.4 The material used shall be in its final finished form and sterilized as indicated for its ultimate use. It shall be handled for implantation in a manner analogous to that for intended final use (for example, special forceps, special cannulas or needles, special syringes, and so forth).

NOTE 5—If this method is used for material research, testing for endotoxin prior to implantation should be considered.

7.5 The candidate material shall be described thoroughly to facilitate development of a suitable implant application protocol. The ADME of the material and its degradation products

should be described. The information shall include, but is not limited to, the following:

7.5.1 Expected mechanism of degradation (for example, hydrolysis, enzymatic, phagocytosis, and so forth).

7.5.2 Expected nonabsorbable degradation products (for example, fibrils, particles from composites).

7.5.3 Expected stages and rate of degradation.

7.5.4 Expected target organ effects (for example, eliminated in the kidney, stored in the liver, stored in the spleen or lymph nodes).

7.6 For each time period, at least six small laboratory animals shall be used with either unilateral or bilateral implants to assess local responses per test and control groups. For larger animals, including rabbits, at least four animals shall be used per time period per test and control group. It is recommended that additional animals be included in the protocol to address assessment of systemic responses (for example, per ISO 10993-11) and to accommodate any differences in *in vitro* and *in vivo* degradation rates of the material.

8. Procedure

8.1 *Implantation:*

8.1.1 Implant the test and control under aseptic conditions in animals that are under surgical plane of anesthesia. For screening studies with subcutaneous implantations, place the articles using a trochar method to avoid the need for an incision. If an incision is needed, insert the implant as far from the incision site as possible. Close the insertion site with a suitable suture material.

8.1.2 The implantation site shall be described and recorded with anatomic landmarks and shall be marked in a manner suitable for identification of the site at the designated time periods. The use of a permanent skin marker and a template marking the placement of the test and the control/sham site is recommended. Articles that are radiopaque may have serial radiographs to identify the location. The implantation of a nonabsorbable marker material such as a monofilament, non-absorbable suture attached to the article or embedded in the gel or liquid is also acceptable. If an implanted marker material is used with the test site, this marker material shall be included in the control/sham site.

8.1.3 Keep the animals in standard housing according to current animal care and use requirements, policies, and regulations. The individual animals should be marked for identification.

8.2 *Post-Operative Care:*

8.2.1 Care of the animals shall be in accordance with accepted standards as outlined in Guide for Care and Use of Laboratory Animals[10] and according to the local and national government ordinances in an approved facility.

8.2.2 Carefully observe each animal during the specified time period and record all relevant observations, including any abnormal clinical findings.

8.2.3 If infection or accidental injury of the test implant site occurs, record the information and process the implant site and tissues and organs as described in 8.3 and 9.1. Exclusion of this data in the final analysis shall be justified, as infection/injury could be implant-related. A replacement animal may be added, if desired.

8.2.4 If an animal dies or is euthanized before the scheduled termination, record the information and process the implant site and tissues and organs as described in 8.3 and 9.1. Exclusion of this data in the final analysis of results shall be justified, as death could be implant-related. The cause of death shall be investigated and reported. If the death is related to anesthesia, a replacement animal may be selected.

8.3 *Euthanasia and Post-Mortem Assessments:*

8.3.1 The euthanasia method shall be the one recommended for the particular animal species according to local and government regulations. The termination time points shall be based on the expected degradation rate of the implant, and include early, intermediate, and late stages of degradation, to include when healing in response to the device or an acceptable steady-state biological response is expected.

NOTE 6—For devices such as orthopedic fracture fixation devices, healing of the fracture may occur prior to device absorption. The duration of the study should include evaluation of the local tissue responses to device absorption.

8.3.2 Termination time points shall be estimated from *in vitro* (for example, real-time or accelerated degradation) or mathematical modeling studies of degradation (for example, based on prior *in vitro* and/or *in vivo* studies), and shall be justified. The early assessment should be conducted when there is no degradation or minimal degradation. The intermediate assessment(s) should be conducted to allow evaluation of histological responses when the device is undergoing degradation, and when the tissue response is expected to be more pronounced based on the degradation profile (for example, increased degradation rate). The late assessment shall include when complete device absorption has occurred, or when a steady-state biological response has been achieved after significant device degradation and additional degradation is not expected to result in adverse biological responses (for example, if an *in vitro-in vivo* correlation (IVIVC) for degradation has been established). Additional assessments should be considered if, at the established assessment time point, the expected degree of degradation or absorption (as estimated by histopathologic assessment) or tissue healing has not occurred. The additional animals recommended in 7.6 may be used for this purpose of additional euthanasia times. See also Clause 5.3.3 of ISO 10993-6:2016.

8.3.3 At euthanasia, record the general appearance of the skin or other tissue at the implantation site. Then, carefully expose the region of the initial implantation. This is facilitated by the use of a template and skin marker at surgery. If a marker suture is used, the site of the marker suture shall be noted. Record the color and consistency of the tissues in the region of the original site of the material. The use of gross photography of the implantation site, when possible, should be considered carefully since it may aid in maintaining an adequate permanent record. Remove the intact tissue envelope around the

[10] National Research Council Guide for the Care and Use of Laboratory Animals, 8th ed (2011). Institute of Laboratory Animal Research Division on Earth and Life Sciences, Washington, D.C. National Academies of Science Press. (http://www.nap.edu/catalog.php?recprdid=12910).