Designation: F1983 – 14

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 will vary depending on chemical composition implant size, implant location, and test subject species; therefore, the implantation times for examination of tissue response will be linked to the rate of absorption. No single implantation time is indicated in this practice.

1.3 These protocols assess the effects of the material on the animal tissue in which it is implanted. The experimental protocols do not fully assess systemic toxicity, carcinogenicity, teratogenicity, 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, all toxicological findings should be recorded. There are some aspects of systemic toxicity, including effects of degradation products on the target organs, that can be addressed with this practice, and these effects should be documented fully.

1.5 *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]

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

F750 Practice for Evaluating Material Extracts by Systemic Injection in the Mouse

F763 Practice for Short-Term Screening of Implant Materials

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

F1408 Practice for Subcutaneous Screening Test for Implant Materials

F1903 Practice for Testing For Biological Responses to Particles ***In Vitro***

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

F1905 Practice For Selecting Tests for Determining the Propensity of Materials to Cause Immunotoxicity (Withdrawn 2011)[3]

F1906 Practice for Evaluation of Immune Responses In Biocompatibility Testing Using ELISA Tests, Lymphocyte Proliferation, and Cell Migration (Withdrawn 2011)[3]

3. Summary of Practice

3.1 Under strict aseptic conditions, specimens of the sterile final implant form candidate material are implanted into the most relevant anatomical tissue site in small laboratory animals, preferably mice, rats, hamsters, or rabbits.

[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.

[3] The last approved version of this historical standard is referenced on www.astm.org.

3.2 The use of larger animals, such as the dog, goat, or sheep may be justified based upon special considerations of the particular study. Choice of species also should 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.

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

4. Significance and Use

4.1 This practice is a guideline for a screening test for the evaluation of the local tissue response to materials that may be selected for implantation into the human body and which are expected to undergo absorption within three years.

4.2 This practice is similar to that for studies on candidate materials 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.

4.3 The material to be tested should be in the final finished form as for intended use, including sterilization. Material/body ratios should be relevant to that of intended device use. Material surface area or mass to body mass ratios of 1X, 10X, and 50X if applicable, are recommended.

4.4 Materials that are designed for use in devices with *in situ* polymerization shall be introduced in a manner such that *in situ* polymerization occurs. Testing of individual precursor components is not recommended.

5. Test Animals and Sites

5.1 Choice of test animal shall take into consideration the normal life span of the animal and the length of the implantation study. Small laboratory animals are preferred. The strain, sex, age, and origin of the animals used should be noted. If larger animals are used, justification for their use should be provided. The source of the animals, species/strain, weight, age (where known or approximate if not known), general health, and boarding conditions should be recorded. Animal use and care regulations shall be followed.

5.2 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 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 similarly sized rodents 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.

5.3 Before embarking on studies in large animals, it is recommended that a pilot study *in vitro* or in rodents be undertaken to determine the expected rate of degradation and assist in the selection of study periods in long-term animal studies. During analysis of study results, the distribution and metabolism of the degradation products should be determined by available analytical methods, such as mass spectrometry. Alternatively, prediction may be done by radio-labeling the material and following the loss of radioactivity; however, radioactive specimens shall not be used for biocompatibility testing. Other methods of characterizing the absorption are acceptable. The target organs of the metabolism and excretion of the products should be identified. It is recommended that acute systemic studies with material extracts according to Practice F750 be completed prior to the initiation of the implantation study.

6. Implant Specimens

6.1 *Design of the Implant*—Specimens may be made from the final finished form candidate material in configurations specific for the animal study. As described in 4.3, the material/host ratio should be available and referrable to ultimate use in the human with material/body mass ratios of 1X, 10X, and 50X, if applicable, recommended. Relevant configurations of implant specimens, such as cylinders, flat cloth, amorphous gels, and polymerizable liquids may be used.

6.2 The implantation site of the candidate material shall be accompanied by the use of an implanted marker or other permanent method, such as a template, to mark the implant site to allow identification of the implant site at the various time periods. In additional animals, control materials shall be implanted by the same techniques, to allow the comparison of the tissue response. When assessing systemic endpoints, it is essential that separate groups of animals be used for test and comparator groups. A sham surgical site, or a sham surgical animal, is necessary.

6.3 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.

6.4 The candidate material shall be described thoroughly to facilitate development of a suitable implant application protocol. The absorption, distribution, metabolism, and excretion of the material and its degradation products should be described. The information shall include, but is not limited to, the following:

6.4.1 Expected method of degradation, for example, hydrolysis, enzymatic, phagocytosis, and so forth.

6.4.2 Expected nonabsorbable degradation products, for example, fibrils, particles from composites.

6.4.3 Expected rate of degradation.

6.4.4 Expected target organ effects where known or expected, for example, eliminated in the kidney, stored in the liver, stored in the spleen or lymph nodes.

6.5 For each time period, at least six rodents shall be used with either single or bilateral implants. For the larger animals, at least four animals shall be used per time period. It is recommended that additional animals be included in the initial

protocol to accommodate any unexpected changes in degradation rates of the material.

7. Procedure

7.1 *Implantation:*

7.1.1 Implant the specimen under sterile conditions in anesthetized animals. Where possible, implant the specimen 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.

7.1.1.1 A sham site or sham animal with the identical implantation procedure, but not the test material, should be included in the protocol. If animals are to be used as part of a systemic toxicity study, the sham shall be a separate animal.

7.1.2 The implantation site 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 specimen and the sham site is recommended. Specimens that are radiopaque may have serial radiographs to identify the location. The implantation of a nonabsorbable marker material such as a monofilament, nonabsorbable suture attached to the specimen or embedded in the gel or liquid also is acceptable. If an implanted marker material is used with the specimen, this marker material shall be included in the sham site. The test specimen site and the sham site shall be marked.

7.1.3 Keep the animals in standard housing according to current animal protection requirements. The individual animals should be marked for identification.

7.2 *Post-Operative Care:*

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

7.2.2 Carefully observe each animal during the specified time period and record any abnormal clinical findings.

7.2.3 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 7.3 and 8.1. Record the data in the results, but do not use the data in the final analysis of results from the other animals. A replacement animal may be added, if desired.

7.2.4 If an animal dies before the scheduled termination, record the information and process the implant site and tissues and organs as described in 7.3 and 8.1. Record the data, but do not use the data in the final analysis of results from the other animals. If the death is related to anesthesia, a replacement animal may be selected.

7.3 *Euthanasia and Implant Retrieval:*

7.3.1 The euthanasia method shall be the one recommended for the particular animal species according to local and government regulations. Euthanasia times shall be based on the expected degradation rate of the material. The initial euthanasia interval shall be when there is expected to be a 50 % loss of mass or release of 50 % of the degradation products. Additional euthanasia times shall include expected 100 % loss of mass, and when complete healing and return to normal histology is anticipated. It is permissible to establish euthanasia times during the study period if at the established time period expected loss has not occurred, for example, if 50 % loss has not occurred when expected, then the euthanasia time for 50 % loss shall again be estimated. Euthanasia at this additional time period is needed. The additional time frames should be advanced to accommodate this slower-than-expected degradation. The additional animals recommended in 6.5 may be used for this purpose of additional euthanasia times.

7.3.2 At euthanasia, record the general appearance of the skin 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 should be considered carefully since it may aid in maintaining an adequate permanent record. Remove the intact tissue envelope around the marker or template and extend beyond any identifiable remaining candidate material. If the candidate material is not evident at the site, extend the explanation site to include several mm of normal tissue on all sides of the marker material or template mark. If any abnormal tissue is observed elsewhere, this shall be removed for further examination. Transfer the tissue specimen as soon as possible into a fixing agent suitable for further histologic processing. The use of alcohol, formaldehyde, or glutaraldehyde is recommended, but other agents or techniques, such as freezing, may be considered. Reference to Practices F561, F981, and F1408 is encouraged for processing procedures.

7.3.3 Although systemic toxicity is not addressed specifically in this practice, examination of target organs should be conducted to maximize use of the animal. After the implantation site is harvested, the abdominal and thoracic viscera should be examined. The liver, spleen, kidney, local lymph nodes, gonads, and lung should be retained in fixative in case of future need. If any abnormalities are noted, the specimen should be subjected to histologic examination. If the release of particles is anticipated, then the target organs shall be processed in an appropriate manner to preserve the particles as discussed in Practices F1903 and F1904.

7.3.4 It is recommended that tissues from the target organs listed in 7.3.3 be processed for histologic analysis since the data may be useful in evaluation of systemic toxicity. Although this practice does not substitute for systemic toxicity studies (see Practice F750), remote organs should be collected and assessed for toxicological findings to maximize use of the animals. Similarly, blood chemistry and hematology, as well as urine studies, may be done on these animals for inclusion in systemic toxicity analysis. The use of these animals for immunotoxicity studies, as discussed in Practices F1905 and F1906, also may be considered.

8. Histologic Evaluation

8.1 *Histological Preparation:*

8.1.1 In general, the standard methods according to Practices F561, F981, and F1408 should be followed. Standard laboratory practices for histological preparation of the implant/

tissue specimens and staining are used (1-5).[4] The tissue and histologic sections should be examined by qualified personnel.

8.1.2 Preservation of the implant material and the tissue reaction are essential; therefore, the entire explant shall be processed without removal of the candidate material. Solvents that dissolve the candidate material before embedding should be avoided where possible. If the material is such that its hardness precludes sectioning with standard microtomes, then cutting and grinding techniques shall be employed. Conventional embedding in paraffin with standard microtomy is not recommended unless it is shown that the candidate material and surrounding tissue are preserved in the specimen. If it is not possible to avoid dissolving the material during fixation and embedding, then care should be taken to mark the location of the material in the tissue.

8.1.3 Tissue response should be characterized in regard to acute inflammation, chronic inflammation, granulation tissue formation, foreign body reaction, and foreign body giant cell formation. Special attention should be given to any change in the integrity of the form of the material, such as solid or mesh changing to particulate and to corresponding changes in tissue response to the altered form of the specimen. Focal tissue loss, necrosis, and granulomas shall be noted. The tissue reaction to the nonabsorbable marker material also should be noted but analyzed separately. Cell numbers may be determined on a histologic evaluation scale of 0 to +4 with 0 being no cellular reaction and +4 being an extensive or severe reaction.

8.1.4 As the material degrades, it can be anticipated that the form of the material may change, and this may result in an altered cellular response. It is important that both the material form and the tissue response be recorded at each time interval.

[4] The boldface numbers in parentheses refer to the list at the end of this text.

9. Report

9.1 The report shall include the following information:

9.1.1 *Implants*—Describe the implant material, its size, weight, shape and form at implantation, mode of degradation, the material characteristics at degradation (for example, free particles, long fibers, amorphous gel, changes in crystallinity), and difficulty in implantation or explantation.

9.1.2 The sterilization method and the method of handling for implantation shall be recorded.

9.1.3 Describe the animal host used, the age, sex, strain, and weight of the animal. The implantation method used shall be described. The records of examination revealing abnormal clinical signs, infection, or death shall be indicated. The cause of death prior to scheduled euthanasia shall be reported.

9.1.4 The length of implantation time, euthanasia method, retrieval technique, gross observation of tissues and organs, and identification of marker suture should be recorded.

9.1.5 The methods of histologic evaluation and the results of histologic evaluation of the implant site and the target organs shall be described. Histologic analysis shall include evaluation scales for acute inflammation, chronic inflammation, foreign body giant cell formation, and other evidence of foreign body reactions, including necrosis.

9.1.6 The material form at explantation shall be recorded.

9.1.7 The final report should include representative photographs of tissue responses showing progressive absorption of implanted materials.

10. Keywords

10.1 absorbables; biocompatibility; degradables; implantation

APPENDIXES

(Nonmandatory Information)

X1. RATIONALE

X1.1 This practice modifies existing ASTM standards on *in vivo* assessment of tissue responses to implanted solid and porous materials, such as Practices F763, F981, F1408, in order to evaluate the local tissue response to absorbable materials. The test procedures for solid and dense materials have a long history of reproducible and meaningful results.

X1.2 The tissue response to absorbable materials is expected to be different from that of nonresorbable materials, and there is not a long history of reproducible and meaningful evaluation. A fibrous capsule is unlikely to be formed, and the presence of cells actively degrading the material or phagocytizing the degradation products may be noted. In many cases, this may have the appearance of a chronic inflammatory response.

X1.3 It is necessary to extend the implantation time in these studies to assess the tissue response during active degradation of the material, when the material has been absorbed entirely, and when the tissue has healed and returned to normal histology. This time period will vary greatly for various types of materials and may extend to more than a year.

X1.4 Some materials may stimulate lengthy remodeling of bone and other tissues and some may be absorbed so slowly that considerable material may remain at the three years designated in 1.1. Materials that are designed for tissue remodeling and slow degradation may be studied in a similar manner as described herein; however, the endpoint for this evaluation should be the formation of the expected normal tissue at the site rather than complete absorption. For those materials, in which tissue remodeling is expected, the time periods should include at least two time periods. The first is when approximately 50 % integration/remodeling has been achieved and the second when the anticipated final histologic

response of remodeling and healing is achieved. For slowly absorbing materials, at least two time periods should be included. The first is when approximately 50 % loss of mass or release of 50 % of the degradation products has occurred, and the second when the biological response indicates a return to the normal tissue histology, such as, the histologic response of the sham site. For example, calcium phosphate ceramics for bone apposition and remodeling should be considered at 100 % endpoint when remodeling bone of normal appearance is observed at the remaining material.

X1.5 This practice does not address all of the issues of subchronic or chronic systemic toxicity; however, it is recommended that the information obtainable from this study that relates to systemic toxicity be analyzed as such. The number of animals suggested in systemic toxicity studies exceeds the number that are needed for local tissue responses. In some cases, therefore, the animals being evaluated for local tissue responses may be a subset of the chronic systemic toxicity study. Each animal in these studies should be used for the maximum obtainable information.

X1.6 It is recognized that it may be difficult to adequately predict the degradation rate and determine when the 50 % absorption has occurred. Imaging techniques, radiopaque markers, or surgical observation may be permitted, as long as they do not impact on the animal welfare or the anticipated biological response.

X2. NOMENCLATURE OF ABSORBABLE AND RELATED TERMS

X2.1 Synthetic implants fabricated from hydrolysable alpha-hydroxy polyesters have been described as "absorbable" since the first polyglycolide-based sutures were commercialized in the United States in the 1970s. At that time, both poly(glycolide) (DEXON—Davis and Geck) and poly(glycolide-co-lactide) copolymer (VICRYL—Ethicon) based sutures were classified as "Absorbable Surgical Suture" by the United States Pharmacopeia (USP) and the United States Food and Drug Administration (US-FDA), a designation that remains to this day. In contrast with "Nonabsorbable Surgical Suture," synthetic glycolide-lactide and collagen-based sutures undergo hydrolytic and/or enzymatic driven chain scission, generating degradation products that are then absorbed by the body. Since this designation, other terms such as "degradable" and "resorbable" have been used interchangeably to describe absorbable implants, with the prefix "bio-" often applied to all these terms.

X2.2 Based on historical usage and regulatory precedent, this practice preferentially utilizes the term absorb/absorbable/absorption to describe implantable synthetic hydrolysable polymeric devices. These same terms are also applied to natural polymers and metals intended to undergo corrosion in vivo, since any degradation product – be it proteinaceous or ionic – will inherently be absorbed by the host organism. The prefix "bio" is avoided since it is redundant in the context of implant applications. "Resorb" and its derivatives are avoided since they are accepted medical terms routinely utilized to describe natural resorption processes present in dynamic tissue, such as osteoclastic driven bone remodeling. "Degrade" and its various derivatives are avoided when referring to either an implantable device or a raw material since common utilization is routinely applied broadly to include composting and other natural processes (including ultra-violet radiation) that cause materials to either intentionally or unintentionally break down into chemical or particulate matter, or both. However, use of the term "degrade" and its derivatives is considered acceptable when referring to breakdown processes (e.g., chain scission, corrosion) within the absorbable materials or implantable device or polymer (for example, "The absorbable implant degrades through hydrolysis" or "During extrusion, absorbable polyglycolide is prone to thermal degradation").

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