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Standard Guide for Pre-clinical *in vivo* Evaluation in Critical Size Segmental Bone Defects[1]

This standard is issued under the fixed designation F 2721; 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 guide covers general guidelines for the *in vivo* assessment of tissue engineered medical products (TEMPs) intended to repair or regenerate bone. TEMPs included in this guide may be composed of natural or synthetic biomaterials (biocompatible and biodegradable) or composites thereof, and may contain cells or biologically active agents such as growth factors, synthetic peptides, plasmids, or cDNA. The models described in this guide are segmental critical size defects which, by definition, will not fill with viable tissue without treatment. Thus, these models represent a stringent test of a material's ability to induce or augment bone growth.

1.2 Guidelines include a description and rationale of various animal models including rat (murine), rabbit (leporine), dog (canine), goat (caprine), and sheep (ovine). Outcome measures based on radiographic, histologic, and mechanical analyses are described briefly and referenced. The user should refer to specific test methods for additional detail.

1.3 This guide is not intended to include the testing of raw materials, preparation of biomaterials, sterilization, or packaging of the product. ASTM standards for these steps are available in the Referenced Documents (Section 2).

1.4 The use of any of the methods included in this guide may not produce a result that is consistent with clinical performance in one or more specific applications.

1.5 Other pre-clinical methods may also be appropriate and this guide is not meant to exclude such methods. The material must be suitable for its intended purpose. Additional biological testing in this regard would be required.

1.6 The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard.

1.7 *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.*

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2. Referenced Documents

2.1 *ASTM Standards:*[2]

ASTM F2721-09

F 561 Practice for Retrieval and Analysis of Medical Devices, and Associated Tissues and Fluids

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F 565 Practice for Care and Handling of Orthopedic Implants and Instruments

F 895 Test Method for Agar Diffusion Cell Culture Screening for Cytotoxicity

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

F 1983 Practice for Assessment of Compatibility of Absorbable/Resorbable Biomaterials for Implant Applications

F 2150 Guide for Characterization and Testing of Biomaterial Scaffolds Used in Tissue-Engineered Medical Products

F 2451 Guide for ***in vivo*** Assessment of Implantable Devices Intended to Repair or Regenerate Articular Cartilage

2.2 *Other Documents:*

ISO 10993 Biological Evaluation of Medical Devices—Part 5: Tests for *in vitro* Cytotoxicity[3]

21 CFR Part 58 Good Laboratory Practice for Nonclinical Laboratory Studies[4]

21 CFR 610.12 General Biological Products Standards—Sterility[4]

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

[4] Available from U.S. Government Printing Office Superintendent of Documents, 732 N. Capitol St., NW, Mail Stop: SDE, Washington, DC 20401, http://www.access.gpo.gov.

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3. Terminology

3.1 *Definitions:*

3.1.1 *bone regeneration*—the formation of bone that has histologic, biochemical, and mechanical properties similar to that of native bone.

3.1.2 *bone repair*—the process of healing injured bone through cell proliferation and synthesis of new extracellular matrix.

3.1.3 *compact bone*—classification of ossified bony connective tissue characterized by the presence of osteon-containing lamellar bone. Lamellar bone is highly organized in concentric sheets.

3.1.4 *cortical bone*—one of the two main types of osseous tissue. Cortical bone is dense and forms the surface of bones.

3.1.5 *critical size defect*—a bone defect, either naturally occurring or artificially created, which will not heal without intervention. In the clinical setting, this term applies to exceeding a healing period of approximately 6 months (in otherwise healthy adults).

3.1.6 *diaphyseal*—pertaining to the mid-section of long bones.

3.1.7 *endochondral ossification*—one of the two main types of bone formation, where a cartilaginous matrix forms first and is subsequently replaced by osseous tissue.

3.1.7.1 *Discussion*—Endochondral ossification is responsible for much of the bone growth in vertebrate skeletons, especially in long bones.

3.1.7.2 *Discussion*—The other main mechanism for bone formation is *intramembraneous ossification*, where osseous tissue is formed directly, without cartilaginous precursor; occurs mainly in the formation of flat bones (skull).

3.1.8 *growth plate*—the anatomic location within the epiphyseal region of long bones corresponding to the site of growth of bone through endochondral ossification.

3.1.8.1 *Discussion*—The growth plate in skeletally mature animals is fused.

3.1.9 *long bone*—bone that is longer than it is wide, and grows primarily by elongation of the diaphysis. The long bones include the femurs, tibias, and fibulas of the legs, the humeri, radii, and ulnas of the arms, the metacarpals and metatarsals of the hands and feet, and the phalanges of the fingers and toes.

3.1.10 *marrow*—soft, gelatinous tissue that fills the cavities of the bones. It is either red or yellow, depending upon the preponderance of hematopoietic (red) or fatty (yellow) tissue.

3.1.10.1 *Discussion*—Red marrow is also called myeloid tissue.

3.1.11 *matrix*—a term applied to either the exogenous implanted scaffold or the endogenous extracelluar substance (otherwise known as extracellular matrix) derived from the host.

3.1.12 *metaphyseal*—pertaining to the dense end-section of long bones.

3.1.13 *remodeling*—a life long process where old bone is removed from the skeleton (bone resorption) and new bone is added (bone formation).

3.1.14 *residence time*—the time at which an implanted material (synthetic or natural) can no longer be detected in the host tissue.

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3.1.15 *skeletal maturity*—the age at which the epiphyseal plates are fused.

3.1.15.1 *Discussion*—In rodents, skeletally mature animals are characterized by defined gonads.

3.1.16 *trabecular bone*—ossified bony connective tissue characterized by spicules surrounded by marrow space.

3.1.17 *weight-bearing versus non-weight bearing models*—weight bearing is the amount of weight a patient or experimental animal puts on the leg on which surgery has been performed, generally described as a percentage of the body weight.

3.1.17.1 *Discussion*—Non weight bearing means the leg must not touch the floor (i.e., supports 0 % of the body weight).

3.1.17.2 *Discussion*—Full weight bearing means the leg can carry 100 % of the body weight on a step.

4. Significance and Use

4.1 This guide is aimed at providing a range of *in vivo* models to aid in preclinical research and development of tissue-engineered medical products (TEMPs) intended for the clinical repair or regeneration of bone.

4.2 This guide includes a description of the animal models, surgical considerations, and tissue processing as well as the qualitative and quantitative analysis of tissue specimens.

4.3 The user is encouraged to use appropriate ASTM and other guidelines to conduct cytotoxicity and biocompatibility tests on materials, TEMPs, or both, prior to assessment of the *in vivo* models described herein.

4.4 It is recommended that safety testing be in accordance with the provisions of the FDA Good Laboratory Practices Regulations 21 CFR 58.

4.5 Safety and effectiveness studies to support regulatory submissions (for example, Investigational Device Exemption (IDE)), Premarket Approval (PMA), 510K, Investigational New Drug (IND), or Biologics License Application (BLA) submissions in the U.S.) should conform to appropriate guidelines of the regulatory bodies for development of medical devices, biologics, or drugs, respectively.

4.6 Animal model outcomes are not necessarily predictive of human results and should, therefore, be interpreted cautiously with respect to potential applicability to human conditions.

5. Animal Models

NOTE 1—This section provides a description of the options to consider in determining the appropriate animal model and bone defect size and location.

NOTE 2—Research using these models needs to be conducted in accordance with governmental regulations and guidelines appropriate to the locale for the care and use of laboratory animals. Study protocols should be developed after consultation with the institutional attending veterinarian, and need appropriate review and approval by the institutional animal care and use committee prior to study initiation.

5.1 *Defect Size*:

5.1.1 A high proportion of fracture injuries in humans occur in long bones. Accordingly, defects created in long bones are commonly used for assessing bone repair/regeneration in animal models.

5.1.2 In principle, critical-size defects may be achieved in both metaphyseal and diaphyseal locations. For the purpose of this guide, only defects created in the diaphyseal section of long bones will be described.

5.1.3 Significant variability exists between animal species with respect to the size and weight of the animal, anatomy, and gait thereby influencing kinetics, range of motion, and mechanical forces on defects. These factors influence bone architecture and structure. These factors play a significant role in the response to injury or disease of bone. The user should consider carefully the animal model that is appropriate for the stage of investigation of an implanted TEMP.

5.1.4 Mechanical load has been shown to affect bone repair. Amongst the mechanobiological factors, intermittent hydrostatic pressure and load-bearing stresses play an important role in modulating bone development and maintenance, as well as bone degeneration The impact of mechanical load extent or duration on the implanted TEMP, and surrounding native bone, varies depending on the anatomic site. The defect site chosen to evaluate implants should, therefore, factor the impact of mechanical load on the performance of the implant.

5.1.5 It is recommended that an appropriate species and anatomic site be chosen, that have dimensions sufficiently large to adequately investigate and optimize the formulation, design, dimensions, and associated instrumentation envisaged for human use, especially in late stages of development.

5.1.6 Larger animals may be more appropriate for studying repair in defects and locations that more closely approximate those in humans.

5.1.7 Larger defect dimensions generally require a method of fixation to secure the implant and thereby reduce implant dislocation. The method of implant immobilization can negatively impact both the surrounding host tissue and repair tissue. Accordingly, the difference in the design of the test TEMP in models which generally do not require fixation should be factored into the interpretation of results with respect to predictability of outcomes in larger animal models and humans requiring fixation.

5.1.8 For each species, a critical size defect is defined as the minimum defect dimension that the animal is incapable of repairing without intervention. The dimensions of critical defects generally differ for each species and should be considered carefully when designing the implant dimensions and method of fixation. As an empirical rule, the length of the defect (created by ostectomy) should at least be equal to 1.5 times the diameter of the selected bone **(1, 2)** .[5] Some authors recommend at least 2 times the diameter of the selected bone **(3)**.

5.1.9 Whether or not the periosteum from the resected segment of bone is still present can influence healing within the bone defect. The periosteum is typically removed in most studies of segmental critical-size defects. Whether or not the periosteum has been removed should be stated when reporting results.

5.1.10 Each study should include an empty-defect control group to confirm that the model is a critical-size defect. If/once the model is very well characterized, the use of historical data instead of actual control animals should be considered, in order to save on animal numbers, unless this would compromise the objectives of the study. For example, in pivotal preclinical proof-of-concept studies, concurrent controls are likely to be appropriate.

5.1.11 The use of unilateral defect models is generally recommended. This is especially true for weight-bearing locations in animals that use all four limbs for weight bearing (especially goats, sheep, and horses).

5.2 *Handling*:

5.2.1 Exposure of implants to extreme and highly variable mechanical forces as a result of jumping and running can lead to increased variability in outcome measures.

5.2.2 Potential differences in outcome when using weight-bearing versus non-weight bearing models should be carefully considered.

5.3 *Chromosomal Sex*:

5.3.1 Due to the impact of circulating steroids on cartilage and bone metabolism and regeneration, the choice of chromosomal sex should be considered. Animals in lactation should not be used. For some purposes, the use of aged or ovariectomized females (especially rats) may be indicated to simulate osteoporotic conditions.

5.3.2 It is recommended that the chromosomal sex be the same within the cohort, and that needs to be reported. The investigator should be aware that variances can occur between sexes and that appropriate statistical power needs to be instituted.

5.4 *Age*:

5.4.1 Bone undergoes dynamic changes in metabolism and remodeling during growth. Due to the impact of these physiologic processes on tissue repair, skeletally mature animals should be used. The cohorts should have fused epiphyseal growth plates.

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[5] The boldface numbers in parentheses refer to the list of references at the end of this standard.

Skeletal maturity varies between species and can be determined radiographically if necessary.

5.4.2 Older animals have a greater propensity for osteopenia and have a decreased capacity to repair bone defects. If specific conditions are considered important for the intended TEMP assessment, then an appropriate model should be used.

5.4.3 The mesenchymal stem cell pool, growth factor responsiveness, and metabolic activity of cells generally decreases with age. Thus, reparative processes that are dependent on the number and activity of native cells may be partially compromised in older animals.

5.5 *Diet or Concurrent Pathology* :

5.5.1 In general, studies are performed with healthy animals under normal diet conditions. However, the addition of fluoride, as well as deprivation of vitamin D and/or calcium have been reported to mimic specific bone disease states. In situations where treatment of patients with systemic conditions that may affect bone repair are contemplated, non-clinical models that mimic the disease or conditions under consideration may be appropriate.

5.6 *Study Duration*:

5.6.1 The length of the study depends on the stage of TEMP development, the species used, the size of the defect, and the composition and design of the implant.

5.6.2 In rats and rabbits, small defects implanted for 8 to 12 weeks provide information regarding the residence time of the implant and fixation of the TEMP as well as the type of repair.

5.6.3 Using larger animals (dogs, sheep, goats), study periods of 8 to 12 weeks are limited to providing information regarding the biocompatibility, early cellular responsiveness, and the persistence and condition of the implant within the defect.

5.6.4 Periods of more than 3 months are generally necessary to gain confidence in the extent of success in the repair or regeneration of bone based on histologic outcome measures.

5.6.5 Depending on the study objective, it might be advisable to evaluate one or more cohorts in the study before full healing occurs. This may be of interest when comparing a new material with a standard material like autograft, where the difference between treatment groups may reach a transient maximum and then diminish over time. In general, it is necessary to match the claim and study end, taking into consideration the statistical power.

5.7 *Number of Animals*:

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5.7.1 A statistically significant number of animals per group needs to be used. The required number depends on the intrinsic variability among the animals being used, the consistency of the surgical procedure which will be performed, the accuracy of the evaluation methods, anticipated attrition rate of animals during the study, and the statistical techniques which will be used to analyze the data **(3)**. Another important factor may be the objective of the study (for example, general feasibility/efficacy compared to an empty defect, or comparability of different constructs), and the variability of the treatment (for example, load of cells/factors, implant dimensions). The group size can be determined from existing data if the respective model is well established (literature or results from preliminary studies). For a pilot study, a group size of 6 to 8 is likely appropriate for histologic and mechanical testing as evaluation methods **(3)**. For group sizes reported in the literature, see Appendix X1.

5.8 *Rat Model*:

5.8.1 Rats are among the most commonly used species for early-phase development, due to relatively low cost, housing space and ease of maintenance. The most commonly used model is a femoral defect **(3)**.

TABLE 1 Most Common Animal Model Parameters for the Assessment of Bone Repair in Critical Size Defects[A]

Species	Breed Commonly Used	Age of Adult Equivalency	Defect Sites Commonly Used	Typical Critical Size Defect Dimensions (mm)	Method of Fixation	Typical End Time-points	Evaluations
Rat[B](Murine)	Sprague-Dawley, athymic nude, Fischer, Wistar, Lewis	6 months	F	5–10 mm	Polyethylene/ polyacetal plate with K-wires/ screws	8–24 weeks	Histology, radiographs/ Faxitron, biomechanics
Rabbit[B] (Lepus)	New Zealand White	9 months	R, U	20 mm	None (radius or ulna left intact)	8–12 weeks	Histology, radiographs, torsional strength
Dog[C] (Canine)	Beagle, Hound, Mongrel	>1–2 years	R, U, F	21–25 mm	Ex-fix, plate/ screws	12–24 weeks	Histology, radiographs, torsional strength
Goat[C] (Caprine)	Swiss Mountain	2–3 years	T	26–35 mm	Ex-fix	26 weeks	Histology, radiographs, compressive strength
Sheep[C] (Ovine)	Merino, Pre-Alpes, other	2–3 years	T, M	25–50 mm	Ex-fix, plate/ screws, intramedullary nail	16–24 weeks	Histology, radiographs, torsional or compressive strength

[A] For citations summarized in table, reference sections 5.7-5.11.
[B] Small animal.
[C] Large animal.

Legend: F, femur; T, tibia; R, radius; U, ulna; M, metatarsal

5.8.2 Since the femur is a load-bearing location, the defect must be stabilized by internal or external fixation. Due to the small size of the animal, the fixation system may have to be custom-made. Plates (polymer, or metal) have been used with screws, K-wires/pins, and/or cerclage wire. Alternatively, external fixators have been described.

5.8.3 The typical defect size is 5 mm, created by a mid-diaphyseal ostectomy using a saw or dental burr. Care has to be taken to not injure the sciatic nerve during the procedure, as disuse of the operated leg can lead to delayed healing of the defect. For more details, see Appendix X1.

5.9 *Rabbit Model*:

5.9.1 The use of rabbits is generally more economical compared to larger species (dogs, sheep, or goats).

5.9.2 The thickness of cortical bone in rabbits is relatively less than in other species included in bone defect evaluations.

5.9.3 The rabbit radius is tubular, which may make it preferable for radiographic, histological, and mechanical evaluation.

5.9.4 A segmental critical size defect in either the ulna or the radius does not require a fixation, since the other bone will act as a stabilizer.

5.9.5 The typical defect size is 15 to 20 mm. Some studies caution that 15 mm is not large enough.

5.9.6 Adult rabbits with closed growth plates are preferred (more than approximately 20 weeks old). In younger animals, the intact bone of the operated leg can be overloaded, resulting in slipping of the growth plate and consequently exclusion from the study.

5.9.7 There is a certain risk in the rabbit ulna model that the radius can become attached to the defect area (fusion), which has to be considered if mechanical testing is one of the outcome measures. The axis of rotation for torsion testing becomes difficult to reproduce, and cross sectional area measurements are also difficult to make.

5.10 *Dog Model*:

5.10.1 Canines, such as medium-sized (for example, mean 10 to 15 kg) mongrels , beagles, and hounds have been used in critical-size defect models **(1, 2, 4-6)**.

5.10.2 Long bones studied in canines have historically included the ulna, radius, and femur **(1, 2, 4-6)**.

5.11 *Sheep Model*:

5.11.1 Sheep are commonly used for the study of bone healing in critical-size long-bone defects in large species animals.

5.11.2 The most common sites in the sheep are the mid-diaphyseal region of the metatarsal **(7-11)** and tibia **(12-18)**.

5.11.3 Defects are typically 2 to 5 cm in the ovine tibia **(7-11)** and approximately 2.5 cm in the ovine metatarsal **(12-18)**.

5.11.4 Defects in the sheep model are typically created unilaterally. Bilateral segmental diaphyseal defects in sheep are strongly discouraged.

5.12 *Goat Model*:

5.12.1 In comparison to sheep, goats are generally less averse to human interaction and are therefore easier to handle.

5.12.2 Goats should be screened by blood test for caprine encephalitis prior to inclusion in the cohort .

5.12.3 Critical-size defects in goats have been created unilaterally in the tibia **(19)**. Bilateral segmental diaphyseal defects in goats are strongly discouraged.

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6. Considerations for Defect Site

6.1 The focus of this guide is on mid-diaphyseal segmental defects in long bones.

6.2 Typical bones for the creation of mid-diaphyseal defects are the ulna, radius, tibia, fibula, and femur. Not all sites have been reported for all species.

6.3 Considerations should also include the level of difficulty of performing the surgical procedure and fixation.

6.4 Bilateral models of critical size segmental defects are generally considered contraindicated due to humane reasons and also possible effects on data integrity.

7. Considerations for Defect Type, Implant Fixation, and Joint Immobilization

7.1 *Joint Loading and Immobilization* :

7.1.1 The animal joint anatomy and joint size as well as gait should be taken into account to determine the appropriate immobilization modality.

7.1.2 Splints, external fixators, and casts can be used to reduce joint motion and loading for variable periods following surgery. There should be a point when the joint is restored to normal activity and exhibits unrestricted motion for an appropriate period of time.

7.1.3 The impact of disuse atrophy and potentially negative consequences to the bone should be considered when choosing the period of immobilization.

7.1.4 Continuous passive motion has been shown to provide some level of benefit to the regenerative process following bone injury in humans and animals. Implementation of similar therapeutic modalities in animal models is less feasible and has not been widely accepted.

7.1.5 The impact of limited access to surgical incision sites associated with the use of casts and splints should be factored into the postoperative care regime.

8. Test Procedures

8.1 *Implant Preparation*:

8.1.1 All materials to be implanted into animals should be verified to be non-cytotoxic and biocompatible. Implant components can be sterilized and prepared aseptically or end-point sterilized by methods known to be acceptable to the implant composition and function.

8.1.2 Bioburden or sterility testing, as appropriate, should be completed on representative test articles. Note that for TEMPs regulated as biologics in the United States, each lot must be tested for sterility in accordance with 21 CFR 610.12.

8.1.3 See Guide F 2150, Practices F 1983, F 981, F 565, and Test Method F 895. Practice F 1983 covers the assessment of compatibility of absorbable biomaterials for implant applications.

8.2 *Defect Generation*:

8.2.1 The defect should be created in a standard and reproducible manner.

8.2.2 Templates or other sizing tools should be considered, where feasible, for preparation of consistently-sized defects.

8.2.3 Defects in all animals within a study should be created with the same type of tools and instruments.

8.3 *Test TEMP Implantation and Fixation*:

8.3.1 The test TEMP should be implanted in a standard and reproducible manner.

8.3.2 Care should be exercised to ensure that the surrounding bone is not excessively damaged and that the TEMP is in contact with the adjacent walls of the defect.

8.3.3 The defect should be fixed in a standard and reproducible manner.

8.4 *Recovery and Husbandry*:

8.4.1 Recovery conditions should be designed to reduce the potential for stress and excessive motion. For goats, sheep, and horses recovery pens that are sized to reduce excessive range of mobility for a period of two to three days are recommended.

8.4.2 All housing conditions should be approved by the United States Department of Agriculture (USDA), or the respective governmental agency of the country where the study is conducted.

8.4.3 Animals should be monitored frequently and observations recorded to ascertain appropriate health and physical condition.

8.4.4 A veterinarian should approve the health condition of animals prior to returning them to larger groups or herds.

8.5 *In-Life Period*:

8.5.1 The use of splints rather than standard dressings can reduce joint motion and loading; however, the impact of disuse atrophy and potentially negative consequence to the bone healing should be considered when choosing the length of treatment.

8.5.2 Radiographs should be used as appropriate for a given study to assess placement of the implants.

8.5.3 Following recovery, large animals should be contained within protected stalls for a minimum of nine days. After this period the animals can either remain in protected stalls or be allowed to roam freely in group herds.

8.5.4 A qualified veterinarian should examine animals routinely for any gross abnormalities or signs of discomfort.

8.5.5 Survival time should be designated based on the objective of the study. Typically, an early timepoint (for example, to examine the effect on early healing, including, for example, acceleration of healing), and one or two later timepoint(s) (for example, when full or nearly full healing is anticipated) are chosen. Historically used in-life periods are listed in the tables in Appendix X1.

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8.6 *Necropsy*:

8.6.1 Animals should be euthanized in a humane manner according to accepted practices of the Animal Welfare Act (in the U.S.) or other applicable local statutes..

8.6.2 The implanted site should be removed along with the surrounding cartilage and bone.

8.6.3 Retrieved tissue should be placed in a solution consistent with intended outcome measures such as histology (decalcified paraffin versus nondecalcified plastic embedded), biochemistry, or mechanical testing.

9. Evaluation and Results

9.1 *Histology*—For histologic processing procedures, refer to Practice F 561. Histological sections should be used to assess the amount and quality of tissue regeneration or repair within the defect. Histologic sections should be serially cut and stained in such a manner as to allow assessment of the quality of tissue and for detection of calcified tissue. Standard stains include: hematoxylin/eosin, Toluidine Blue, Modified Trichrome stain, and others **(3)**. Consideration should be given to using decalcified versus undecalcified sections, which may require different staining methods.

9.1.1 *Microscopic Analysis and Scoring* :

9.1.1.1 Histologic sections should be analyzed for adverse tissue reactions using histopathologic indices.

9.1.1.2 For assessment of TEMP performance, a scoring system should be used to determine several aspects such as the following: callus formation, new bone formation in the defect (mineralized/non-mineralized), resorption of bone graft, cortex remodeling, marrow changes, union (distal, proximal) (for example, Ref **3**). In addition, fibrous connective tissue should be evaluated with regard to inflammation.

9.1.1.3 Histomorphometric analyses can be used to measure histologic parameters such as thickness, integration, cell number, and surface quality.

9.1.1.4 Time points of less than six months do not necessarily reflect the long-term outcome due to the potential for changes in the biochemical composition and organization of repair tissue over time.

9.1.1.5 Short-term histologic evaluation can be used for screening and optimization. Long-term assessment should be based on histologic and mechanical measures.