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Standard Guide for Pre-clinical *in vivo* Evaluation of Spinal Fusion[1]

This standard is issued under the fixed designation F2884; 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 pre-clinical *in vivo* assessment of tissue-engineered medical products (TEMPs) intended to repair or regenerate bone in an interbody and/or posterolateral spinal environment. TEMPs included in this guide may be composed of, but are not limited to, natural or synthetic biomaterials 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 document represent a stringent test of a material's ability to induce and/or augment bone growth in the spinal environment.

1.2 While clinically TEMPs may be combined with hardware for initial stabilization or other purposes, the focus of this guide is on the appropriateness of the animal model chosen and evaluation of the TEMP-induced repair and as such does not focus on issues of components or constructs.

1.3 Guidelines include a description and rationale of various animal models for the *in vivo* assessment of the TEMP. The animal models utilize a range of species including rat (murine), rabbit (lapine), dog (canine), goat (caprine), pig (porcine), sheep (ovine), and non-human primate (primates). Outcome measures include *in vivo* assessments based on radiographic, histologic, and CT imaging as well as subsequent *in vitro* assessments of the repair, including histologic analyses and mechanical testing. All methods are described briefly and referenced. The user should refer to specific test methods for additional detail.

1.4 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 Referenced Documents (Section 2).

1.5 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.

1.6 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.

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

1.8 The values stated in inch-pound units are to be regarded as standard. The values given in parentheses are mathematical conversions to SI units that are provided for information only and are not considered standard.

1.9 *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

F565 Practice for Care and Handling of Orthopedic Implants and Instruments

F895 Test Method for Agar Diffusion Cell Culture Screening for Cytotoxicity

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

F1983 Practice for Assessment of Selected Tissue Effects of Absorbable Biomaterials for Implant Applications

F2150 Guide for Characterization and Testing of Biomaterial Scaffolds Used in Regenerative Medicine and Tissue-Engineered Medical Products

2.2 *Other Standards:*

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

[1] This guide is under the jurisdiction of ASTM Committee F04 on Medical and Surgical Materials and Devices and is the direct responsibility of Subcommittee F04.44 on Assessment for TEMPs.

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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.

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

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

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 remodeling*—a lifelong process where old bone is removed from the skeleton (a sub-process called bone resorption) and new bone is added (a sub-process called bone formation).

3.1.2.1 *Discussion*—These processes also control the re-shaping or replacement of bone during growth and following injuries. Remodeling responds to functional demands and muscle attachments. As a result, bone is added where needed and removed where it is not required.

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

3.1.4 *cancellous bone*—(also known as trabecular, or spongy, bone), a type of osseous tissue with a low apparent density and strength but very high surface area, that fills the inner cavity of long bones.

3.1.4.1 *Discussion*—The orientation of the trabecular bone is such that the trabecular "struts" tend to follow the lines of stress to which the bones are normally subjected. The external layer of cancellous bone contains red bone marrow where the production of blood cellular components (known as hematopoiesis) takes place. Cancellous bone is also where most of the arteries and veins of bone organs are found.

3.1.5 *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.

3.1.6 *cortical bone*—one of the two main types of osseous tissue; cortical bone is dense and forms the surface of 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; it 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 through endochondral bone formation.

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

3.1.9 *interbody spine fusion*—a method of obtaining spinal fusion that involves placing bone graft between adjacent vertebrae in the area usually occupied by the intervertebral disc.

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 *posterolateral spine fusion*—a method of obtaining spinal fusion that involves placing bone graft in the "gutter" in the posterolateral portion of the spine between the transverse process and the spinous process.

3.1.12.1 *Discussion*—Posterolateral spine fusion is also known as posterolateral gutter spine fusion.

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

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

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 *spinal fusion*—also known as spondylosyndesis, is a surgical technique used to combine two or more vertebrae.

3.1.16.1 *Discussion*—Supplementary bone tissue (either autograft or allograft) is often used in conjunction with the body's natural osteoblastic processes. This procedure is used primarily to eliminate the pain caused by abnormal motion of the vertebrae by immobilizing the vertebrae themselves. Spinal fusion is done most commonly in the lumbar region of the spine, but it is also used to treat cervical and thoracic problems.

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

3.1.18 *vertebra (plural: vertebrae)*—the vertebral column is the individual irregular bones that make up the spinal column (also known as ischis)—a flexuous and flexible column.

3.1.18.1 *Discussion*—There are normally thirty-three (33) vertebrae in humans, including the five that are fused to form the sacrum (the others are separated by intervertebral discs) and the four coccygeal bones which form the tailbone. The upper three regions comprise the remaining 24 and are grouped under the names cervical (seven vertebrae), thoracic (twelve vertebrae), and lumbar (five vertebrae), according to the regions they occupy. This number is sometimes increased by an additional vertebra in one region, or it may be diminished in one region, the deficiency often being supplied by an additional vertebra in another. The number of cervical vertebrae is, however, very rarely increased or diminished. Each vertebra is composed of a body anteriorly and a neural arch posteriorly.

[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.

The arch encloses an opening, the vertebral foramen, which helps to form a canal in which the spinal cord is housed. Protruding from the posterior extreme of each neural arch is a spinous process and extending from the lateral edges of each arch are transverse processes. These bony elements serve as important sites of attachment of deep back muscles. The neural arch of each vertebrae is divided into component parts by these processes. The parts of the neural arch between the spinous and transverse processes are known as the laminae and the parts of the arch between the transverse processes and the body are the pedicles. At the point where the laminae and pedicles meet, each vertebra contains two superior articular facets and two inferior articular facets. The former pair of facets form articulations, which are synovial joints, with the two inferior articular facets of the vertebra immediately above (or the skull, in the case of the first cervical vertebra). The pedicle of each vertebra is notched at its superior and inferior edges. Together the notches from two contiguous vertebra form an opening, the intervertebral foramen, through which spinal nerves pass.

3.1.19 *vertebral body*—the largest part of a vertebra and is approximately cylindrical in shape.

3.1.19.1 *Discussion*—Its upper and lower surfaces are flattened and rough, and give attachment to the intervertebral fibrocartilages, and each presents a rim around its circumference. In front, the body is convex from side to side and concave from above downward. Behind, it is flat from above downward and slightly concave from side to side. Its anterior surface presents a few small apertures, for the passage of nutrient vessels. On the posterior surface is a single large, irregular aperture, or occasionally more than one, for the exit of the basi-vertebral veins from the body of the vertebrae.

4. Significance and Use

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

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 utilize 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.

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 fusion location.

Note 2—Research using these models needs to be conducted in accordance with governmental regulations appropriate to the locale and guidelines 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 Considerations:*

5.1.1 Spinal fusion is typically performed on a patient who has sustained trauma in order to stabilize the spine, to relieve a neural deficit related to bony stenosis, or to treat degenerative disc disease. A high proportion of injuries in humans occur in the spine. Accordingly, defects created in the spine are commonly used for assessing spinal bone repair/regeneration in animal models.

5.1.2 Defects may be created surgically in both the interbody and posterolateral spinal locations. For the purpose of this guide, defects created in both spinal regions 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 TEMPs. Table X1.1 is provided to give guidance for the selection of animal models and the relevancy of their results.

5.1.4 Mechanical load has been shown to affect bone repair. The 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 the amount and duration of the mechanical load on the implanted TEMPs, and surrounding native bone, varies depending on the anatomic site.

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

5.1.6 Spinal interbody surgical procedures generally require a method of stabilization, typically some sort of load-bearing interbody implant. Larger animals may be more appropriate for studying repair in the interbody location due to size constraints associated with applying spinal interbody fusion devices used to provide load support, as well as sizing of appropriate stabilization components or constructs such as spinal rods, plates, and/or screws.

5.1.7 The use of pedicle screw and rod constructs varies in the literature and is dependent upon several factors, including the amount of instability created by the surgery as well as how closely researchers may wish to mimic the human clinical scenario. 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.

5.1.8 In regard to instrumentation, both interbody fusion devices and pedicle screws, there are pros and cons. Pros include the fact that the surgical intervention more closely mimics that of human clinical surgeries. Cons include increased study cost, animal intervention, and surgical time. The use of instrumentation must be balanced against the desired outcomes of the study and the frequency of healing in the particular animal model compared to the human.

5.1.9 Each study should include a control group containing an acceptable standard of care, usually autograft for positive controls or shams for negative controls, to confirm that the model results demonstrate consistency with accepted values for healing. Allograft may also be an option for use in animal models where donor material is from animals of genetically identical strains, for example, athymic (rnu/rnu) rats. In cases where the product being tested consists of a combination of agents (for example, cells and a matrix), each separate component of the combination product should be tested individually as controls, where possible or appropriate. If/once the model is very well characterized and considered "validated," the use of historical data (from published literature or lab studies using an identical "validated" model) 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 pre-clinical proof-of-concept studies, concurrent controls are likely to be appropriate.

5.1.10 For screening materials, small animals (rats, rabbits) are best due to relative cost and a sizeable amount of literature to support their use in posterolateral spine material evaluations for bone fusion.

5.1.11 Larger animals may be more appropriate for studying repair in the interbody location due to size constraints associated with applying interbody spinal fusion devices used to provide load support, as well as sizing of appropriate stabilization components or constructs such as spinal rods, plates, and/or screws.

5.1.12 In TEMPs which use components that depend on a particular dose range in order to function appropriately, the dose ranges should be appropriate for the animal model used. In general, larger animals require doses of material scaled appropriately. Non-human primates are likely the best choice when targeting doses which may potentially approach the ranges of human clinical dose ranges.

5.1.13 Regardless, all animal models contain inherent limitations and these limitations should be noted where possible. Drawbacks may include factors such as more rapid bone healing than observed in humans, relatively small amounts of material that can be implanted, and these models do not reflect the range of pathology (age, osteoporosis, soft tissue injury) or deleterious systemic agents (steroids, malnutrition, smoking) that may be present in humans. Also, differences in loading environments between quadripedal animals and bipedal humans must be considered. In some instances of new intended use and/or new materials, human clinical data may still be necessary.

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 instrumented versus non-instrumented 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 (**1-24**).[5]

5.3.2 It is recommended that the chromosomal sex be the same within the cohort, and 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. 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 decrease 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*—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 to mimic specific bone disease states, has been reported (**13, 21, 25, 26**). In situations where treatment of patients with systemic conditions that may affect bone repair are contemplated, non-clinical models that mimic the disease or condition 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 composition and design of the implant.

5.6.2 Short-term in small animals (rats, rabbits) can be taken to mean less than twelve weeks in life, long-term is twelve to 24 weeks or greater. In large animals (dogs, pigs, sheep, goats,

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

non-human primates) short-term can be considered to mean less than six months in life, and long-term six months or greater.

5.6.3 In small animals, small defects implanted for five to twelve weeks provide information regarding residence time of implant and fixation of the TEMP as well as the type of repair.

5.6.4 Using larger animals, study periods of eight to twelve weeks are limited to providing information regarding the biocompatibility, early cellular responsiveness, and the persistence and condition of the implant within the defect.

5.6.5 Periods of more than three months for mid-size to larger animals are generally necessary to gain confidence in the extent of success in the repair or regeneration of bone based on histologic and biochemical outcome measures.

5.6.6 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*—A statistically significant number of animals per group is recommended to be used, if possible. 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 (**27**). 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 six to eight is likely appropriate for histologic and mechanical testing as evaluation methods (**27**). For group sizes reported in the literature, see the appendix.

5.8 *Rat Posterolateral Spine Model:*

5.8.1 Rats are amongst the most commonly used species for early-phase development, due to relatively low cost, housing space, and ease of maintenance (**28-44**). Often, Sprague-Dawley or athymic rats are used to assess results because the fusions involve human-derived materials (such as demineralized bone products). In cases where autograft or synthetic biomaterials are used, normothymic Sprague-Dawley rats may be used (**32**).

5.8.2 Surgical defects are typically performed at the L4-L5 lumbar level.

5.8.3 For more details, see Appendix X2, Table X2.1.

5.9 *Rabbit Posterolateral Spine Model:*

5.9.1 Rabbits are the most commonly used animal model for spinal posterolateral fusion (**39, 45-142**) assessment due to a variety of factors (cost, model validation work, and so on) and nonunions spontaneously occur at a similar rate as in human (**55, 143**).

5.9.2 Adult rabbits with closed growth plates are preferred (more than approximately 20 weeks old).

5.9.3 Surgical defects are typically performed at the L4-L5 or L5-L6 lumbar levels.

5.9.4 For more details, see Appendix X2, Table X2.2.

5.10 *Dog Posterolateral Spine Model:*

5.10.1 Canines such as medium-size mongrels (for example, mean 10 to 20 kg) and hounds have been utilized in posterolateral spinal models (**144-153**).

5.10.2 Surgical defects are typically performed at one or more of the L2-L3, L3-L4, L4-L5, or L5-L6 lumbar levels.

5.10.3 An average of approximately 2 to 3 g (**145, 149**) or 15 cc (**150**) of the desired graft material is placed at the operative site bilaterally.

5.10.4 For more details, see Appendix X2, Table X2.3.

5.11 *Dog Interbody Spine Model:*

5.11.1 Canines such as medium-size mongrels (for example, mean 10 to 15 kg) and hounds have been utilized in interbody spinal models, mostly in the location of the cervical spine (**154-165**).

5.11.2 Surgical defects are typically performed at one or more of the C3-C4 and C5-C6 cervical levels.

5.11.3 The discs of the chosen levels are excised leaving the posterior longitudinal ligaments intact.

5.11.4 Opposing vertebral cartilaginous endplates are scraped clean with a curette and a high-speed burr.

5.11.5 Care should be taken to produce a flat surface for implant insertion and seating (assuming an impacted-type implant).

5.11.6 The interbody fusion device is packed with the desired TEMP.

5.11.7 Using finger pressure or gentle impaction, the desired interbody fusion device is inserted.

5.11.8 The interbody fusion device is placed such that it is in contact with the anterior cortices.

5.11.9 For more details, see Appendix X2, Table X2.4.

5.12 *Sheep Posterolateral Spine Model:*

5.12.1 Sheep are commonly used for posterolateral spinal fusion studies in large species animals (**166-182**).

5.12.2 Surgical defects are typically performed at one or more of the L2-L3, L3-L4, L4-L5, or L5-L6 lumbar levels.

5.12.3 Ten (10) cc of autogenous cancellous bone may be harvested, if used as a control, per side.

5.12.4 The transverse processes of the operative levels are decorticated bilaterally.

5.12.5 Treatment or control materials are placed along the "gutters" between the transverse processes.

5.12.6 Optionally, transpedicular screw fixation using screws and rods may be used for fixation.

5.12.7 For more details, see Appendix X2, Table X2.5.

5.13 *Sheep Interbody Spine Model:*

5.13.1 Sheep are commonly used for interbody spinal fusion studies in large species animals (**166, 176, 181, 183-213**).

5.13.2 Surgical defects are typically performed at one or more of the L2-L3 or L4-L5 lumbar levels or the C2-C3, C3-C4, C4-C5, or C5-C6 cervical levels.

5.13.3 An interbody fusion device is filled with an appropriate bone graft material and implanted at each disc space.

5.13.4 Optionally, the lumbar fusion sites may be stabilized with unilaterally placed pedicle screws and a connecting rod.

5.13.5 For more details, see Appendix X2, Table X2.6.

5.14 *Goat Posterolateral Spine Model*—Goats have not typically been used for posterolateral spinal fusion studies in large species animals. They have been used to evaluate a variety of bone graft materials using cassettes containing multiple materials for evaluation at a single transverse process site or for studying posterior construct mechanics (**214-218**).

5.15 *Goat Interbody Spine Model:*

5.15.1 Goats are commonly used for interbody spinal fusion studies in large species animals (**219-247**).

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

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

5.15.4 Surgical defects are typically performed at one or more of the L2-L3, L3-L4, L4-L5, or L5-L6 lumbar levels or the C2-C3, C3-C4, C4-C5, or C5-C6 cervical levels.

5.15.5 An interbody fusion device is filled with an appropriate bone graft material and implanted at each disc space.

5.15.6 Optionally, the lumbar fusion sites may be stabilized with unilaterally placed pedicle screws and a connecting rod.

5.15.7 For more details, see Appendix X2, Table X2.7.

5.16 *Pig Posterolateral Spine Model:*

5.16.1 Pigs have been utilized in posterolateral spinal models, although not as frequently in literature as other large animal models (**248-251**).

5.16.2 Surgical defects are typically performed at one or more of the L2-L3, L3-L4, L4-L5, or L5-L6 lumbar levels.

5.16.3 Approximately 4 to 8 g of autogenous cancellous bone may be harvested, if used as a control, per side.

5.16.4 The transverse processes of the operative levels are decorticated bilaterally.

5.16.5 Treatment or control materials are placed along the "gutters" between the transverse processes.

5.16.6 Optionally, transpedicular screw fixation using screws and rods may be used for fixation.

5.16.7 For more details, see Appendix X2, Table X2.8.

5.17 *Pig Interbody Spine Model:*

5.17.1 Pigs have been utilized in interbody spinal models, although not as frequently in literature as other large animal models (**252-273**).

5.17.2 Surgical defects are typically performed at one or more of the L2-L3, L3-L4, L4-L5, or L6-L7 lumbar levels.

5.17.3 An interbody fusion device is filled with an appropriate bone graft material and implanted at each disc space.

5.17.4 Optionally, the lumbar fusion sites may be stabilized with unilaterally placed pedicle screws and a connecting rod.

5.17.5 For more details, see Appendix X2, Table X2.9.

5.18 *Non-Human Primate Posterolateral Spine Model:*

5.18.1 Non-human primates have been utilized in posterolateral spinal models (**274-286**).

5.18.2 Surgical defects are typically performed at the L4-L5 lumbar level.

5.18.3 Approximately 4 g of autogenous cancellous bone may be harvested, if used as a control, per side.

5.18.4 The transverse processes of the operative levels are decorticated bilaterally.

5.18.5 Treatment or control materials are placed along the "gutters" between the transverse processes.

5.18.6 For more details, see Appendix X2, Table X2.10.

5.19 *Non-Human Primate Interbody Model:*

5.19.1 Non-human primates have been utilized in interbody spinal models (**287-294**).

5.19.2 Surgical defects are typically performed at one or more of the L2-L3, L3-L4, L5-L6, or L7-S1 lumbar levels.

5.19.3 An interbody fusion device is filled with an appropriate bone graft material and implanted at each disc space.

5.19.4 For more details, see Appendix X2, Table X2.11.

6. Considerations for the Spinal Fusion Site

6.1 The focus of this guide is on interbody and posterolateral fusion sites in the spine. Not all sites have been reported for all species.

6.2 Considerations should also include the level of difficulty of performing the surgical procedure in regards to both surgical access and implant fixation.

6.3 Consideration should be given to the level of translatability of the surgical procedure to human clinical patients.

7. Test Procedures

7.1 *Implant Preparation:*

7.1.1 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 endpoint sterilized by methods known to be acceptable to the implant composition and function.

7.1.2 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.

7.1.3 See Guide F2150, Practices F1983, F981, F565, and Test Method F895. See also ISO 10993 and 21 CFR Part 58. Practice F1983 covers the assessment of compatibility of absorbable biomaterials for implant applications.

7.2 *Defect Generation:*

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

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

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

7.3 *Test TEMP Implantation and Fixation:*

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

7.3.2 Care should be exercised to ensure that the surrounding bone is not excessively damaged and that the TEMP is in contact with as much of the area of the defect as possible.

7.3.3 The defect should be fixed in a standard and reproducible manner, if fixation is required.

7.4 *Recovery and Husbandry:*

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

7.4.2 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.

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

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

7.5 *In-Life Period:*

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

7.5.2 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.

7.5.3 A qualified veterinarian should examine animals routinely for any gross abnormalities and for signs of discomfort.

7.5.4 Survival time should be designated based on the objective of the study. Typically, an early time point (for example, to examine the effect on early healing, including, for example, acceleration of healing), and one or two later time point(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 X2.

7.6 *Necropsy:*

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

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

7.6.3 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.

8. Evaluation and Results

8.1 *Histology*—For histological processing procedures, refer to Practice F561. Histological sections should be used to assess the amount and quality of tissue regeneration or repair of the fusion mass. Histological sections should be serially cut and stained in a manner to allow for assessment of the quality of tissue and for detection calcified tissue. Standard stains include: hemotoxylin/eosin, Toluidine Blue, or Modified Trichrome stain, and others (**27**). Consideration should be given to using decalcified versus nondecalcified sections, which may require different staining methods.

8.1.1 *Microscopic Analysis and Scoring:*

8.1.1.1 Histological sections should be analyzed for adverse tissue reactions using typical histopathologic indices.

8.1.1.2 For assessment of TEMP performance, a scoring system should be utilized to determine several aspects such as the following: new bone formation (mineralized/nonmineralized) in the defect, resorption of bone graft, cortex remodeling, marrow changes, and spinal fusion. In addition, fibrous connective tissue should be evaluated with regard to inflammation.

8.1.1.3 Histomorphometric analyses can be utilized to measure histological parameters, including (but not limited to) tissue volume, lamellar bone (area, %), periosteal fibrosis (area, %), marrow fibrosis (area, %), and cellularity (number, mean/field).

8.1.1.4 Histological sectioning should ensure that the entire defect site, as well as some additional surrounding tissue, is encompassed and assessed.

8.1.1.5 Note that time points of less than six months for large animals and less than twelve weeks in small animals 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.

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

8.2 *Radiography:*

8.2.1 Radiographs are important to evaluate the amount and quality of the new bone forming during the in-life portion of the study, as well as at the endpoint.

8.2.2 Typically, radiographs should be taken in two orthogonal planes to allow assessment of proper alignment and a quasi-three-dimensional view (for example, anterior-posterior and lateral).

8.2.3 Radiographic healing may be one of the decisive factors used to terminate a study.

8.2.4 Various radiographic scoring systems have been published. The scoring system should be specified for the species.

8.2.5 Inclusion of a metal wedge in the picture may help to normalize radiographs.

8.2.6 Radiopaque implants and fixation materials may have an impact on the ability to assess healing from radiographs.

8.2.7 Plain-film radiographs are not considered to be sufficiently discriminating to positively identify fusion or pseudarthrosis and should be combined with other methods to verify fusion.

8.3 *Computer Tomography:*

8.3.1 Computer tomography (CT) has been evolving in recent years as a useful tool, allowing 3D imaging of bone regeneration in harvested bone, as well as being used for monitoring bone regeneration *in vivo* over time.

8.3.2 CT images to assess bone (mineralized tissue) area are also useful for correct calculation and interpretation of mechanical test results.

8.3.3 The biggest challenge with CT analyses is to threshold appropriately to exclude the scaffold from newly forming bone within the defect.

8.3.4 Appropriate controls, calibrations, and scan parameters (energy intensity, integration time, and so on) should be utilized in order to ensure that the results are internally consistent within a study.

8.3.5 Where fusion versus pseudarthrosis is an outcome measure, CT outcomes should be verified by histology, manual manipulation, or mechanical testing.

8.4 *Microtomography:*

8.4.1 Microtomography, or micro-CT, uses X-rays to create cross sections of a 3D object that later can be used to recreate a virtual model without destroying the original model. The term micro is used to indicate that the pixel sizes of the cross sections are in the micrometer range. Scanners are much smaller in design compared to the human versions and are used to model smaller objects. Micro-CT scanning is more focused than regular CT scanning, meaning that it brings out details as fine as 1000th of a millimeter. Thus, it has two to three thousand times the resolution of a regular CT scan.

8.4.2 Microtomography analysis can be used to assess volume rendering and for image segmentation. Similar to CT, micro-CT images to assess bone (mineralized tissue) area are also useful for correct calculation and interpretation of mechanical test results.

8.4.3 Appropriate controls, calibrations, and scan parameters (energy intensity, integration time, and so on) should be utilized in order to ensure that the results are internally consistent within a study.

8.4.4 Where fusion versus pseudarthrosis is an outcome measure, CT outcomes should be verified by histology, manual manipulation, or mechanical testing.

8.5 *Mechanical Testing of Repair Tissue:*

8.5.1 Mechanical testing of the fusion usually follows dissection. Care has to be taken when separating the spine sections if fusion is observed. Sample preparation may involve partial embedding into resin blocks to allow proper mounting in the fixtures.

8.5.2 Standard nondestructive testing may include manual palpation as an assessment of spinal fusion.

8.5.3 The specific testing apparatus, load cell resolution, loading constraints, loading profile, and other test parameters as required need to be documented.

8.5.4 Typical nondestructive testing includes protocols to determine global and localized range of motion (ROM) and stiffness. Testing typically occurs under either load or displacement control.

8.5.5 Typical destructive testing includes tension testing and torsional strength testing for posterolateral fusion and dynamic cyclic load to failure for interbody fusions.

8.5.6 Due to viscoelastic effects, consideration has to be given to the test speed utilized in static testing, which should be lower than an appropriate % length change for the test, for example 0.5 % strain/min, and reported.

8.5.7 From typical stress-strain curves, the strength (maximum torque), maximum force, stiffness, and total energy to failure can be calculated. From torsional tests, it is necessary to also report the angle at failure. From cyclic load tests, it is necessary to report the frequency and amplitude of the loading, as well as the cycles to failure.

8.5.8 It is recommended to monitor and report where the fracture at failure occurs (in or through the newly formed bone tissue, or in the original bone outside the defect). Faxitron radiographs may be used as a tool for this purpose.

9. Analysis

9.1 *Statistical Analysis*—The mean and standard deviation should be calculated for the individual categories and the total score for each of the graded specimens. Fisher exact test, chi-square test, or Kruskal-Wallis test (a one-way nonparametric analysis of variance) can be used for analyzing the differences between the scores of different groups.

10. Keywords

10.1 animal models; biomaterials; bone; bone regeneration; bone repair; implants; interbody spine fusion; *in vivo*; mechanical testing; pre-clinical; products; posterolateral spine fusion; spinal fusion; spine; synthetic biomaterials; TEMPs (tissue engineered medical products)

APPENDIXES

(Nonmandatory Information)

X1. COMMON ANIMAL MODEL PARAMETERS AND RELEVANCE IN SPINAL FUSION PRE-CLINICAL MODELS

TABLE X1.1 Common Animal Model Parameters and Relevance in Spinal Fusion Pre-Clinical Models[A]

NOTE 1—Literature Search Strategy used PubMed and a keyword search to identify potential articles.

NOTE 2—Search terms used: spine, posterolateral, sheep, pig, dog, non-human primate, monkey, interbody, intradiscal, device, cages, goat, rat, rabbit, animal models, biomaterials, bone, bone regeneration, bone repair, spine, spinal fusion, pre-clinical, interbody spine fusion, posterolateral spine fusion, products, implants, *in vivo*, mechanical testing, synthetic biomaterials, TEMPs (tissue-engineered medical products), murine, lapine, canine, caprine, porcine, ovine, primates.

NOTE 3—Literature cited was chosen in order to be representative of the literature findings for the respective spinal animal models.

Model	Breed Commonly Used	Defect Site	Instrumented	Duration (see 5.6)	Typical Evaluation Methods	Relevance				
						Final Material Testing	Comparative Performance Data	Mechanistic Studies	Screening	Safety Studies
Large animal (Non-human primate, canine, sheep, goats)	**goat**: Swiss Mountain; **canine**: Beagle, Hound, Mongrel; **sheep**: Merino, Pre-Alpes, other; **non-human primate**: Rhesus Macaque (Macaca mulatta), Chacma Baboon (Papio ursinus)	Posterolateral	Yes	Long-term	Histological, radiographs, CT, mechanical (manual palpation)	X	X			X
Large animal (Non-human primate, canine, sheep, goats)	**goat**: Swiss Mountain; **canine**: Beagle, Hound, Mongrel; **sheep**: Merino, Pre-Alpes, other; **non-human primate**: Rhesus Macaque (Macaca mulatta), Chacma Baboon (Papio ursinus)	Interbody	Yes	Long-term	Mechanical, Histological, CT	X	X			X
Large animal (Non-human primate, canine, sheep, goats)	**goat**: Swiss Mountain; **canine**: Beagle, Hound, Mongrel; **sheep**: Merino, Pre-Alpes, other; **non-human primate**: Rhesus Macaque (Macaca mulatta), Chacma Baboon (Papio ursinus)	Posterolateral	No	Long-term	Histological, radiographs, CT, mechanical (manual palpation)	X	X			X
Large animal (Non-human primate, canine, sheep, goats)	**goat**: Swiss Mountain; **canine**: Beagle, Hound, Mongrel; **sheep**: Merino, Pre-Alpes, other; **non-human primate**: Rhesus Macaque (Macaca mulatta), Chacma Baboon (Papio ursinus)	Posterolateral	Yes	Short-term	Histological, radiographs, CT, mechanical (manual palpation)	X	X			X
Large animal (Non-human primate, canine, sheep, goats)	**goat**: Swiss Mountain; **canine**: Beagle, Hound, Mongrel; **sheep**: Merino, Pre-Alpes, other; **non-human primate**: Rhesus Macaque (Macaca mulatta), Chacma Baboon (Papio ursinus)	Interbody	Yes	Short-term	Mechanical, Histological, CT	X	X			X
Small animal (Rabbits, Rats)	**rat**: Sprague-Dawley, athymic nude, Fischer, Wistar, Lewis; **rabbit**: New Zealand White, Japanese White	Posterolateral	No	Long-term	Histological, micro-CT, mechanical (manual palpation)	X	X	X	X	X
Small animal (Rabbits, Rats)	**rat**: Sprague-Dawley, athymic nude, Fischer, Wistar, Lewis; **rabbit**: New Zealand White, Japanese White	Posterolateral	No	Short-term	Histological, micro-CT, mechanical (manual palpation)	X	X	X	X	X

[A] Clinical efficacy can only be determined through human clinical experience. No animal model has been validated to predict actual clinical performance.

X2. PUBLISHED SPINE FUSION PRE-CLINICAL MODEL EXAMPLES

TABLE X2.1 Published Examples for the Rat Posterolateral Spine Fusion Model

Category	Publication Reference			
Citation	Grauer (32)	Bomback (29)	Abe (31)	Hidaka (34)
Breed	Athymic nude rat, normothymic Sprague-Dawley rat	Athymic nude rat	Normothymic Sprague-Dawley rat	Lewis rats
Chromosomal Sex	Female	Not specified	Male	Not specified
Age	8–9 weeks (athymic), 9–10 weeks normothymic	8–9 weeks	8 weeks	Not specified
Weight	Not specified	170–200 g	Not specified	200–300 g
Group Size (n)	N=40 athymic, N=20 normothymic Sprague-Dawley	N=30/group (60 total)	N=40, N=42 (82 total)	4 groups (9, 10, 11, 12, 12); (54 total)
Intertransverse Location	L4-L5	L4-L5	L4-L5	L4-L5
Control	No graft	None	None	Fresh bone graft from syngeneic Lewis rats
Bone Graft Volume	0.1–0.2 cc per side	2 cc/kg (~0.2 cc per side)	0.2 g per side	50 mg/site
Bone Graft Material	Autograft	Grafton Putty or OP-1 Putty	Autograft (with saline or with subcutaneous PTH injections)	Freeze-dried allograft bone (50 mg/site) and genetically modified syngenic bone marrow cells (1.5×10^6/site) suspended in 50:l type 1 collagen gel (2 mg/mL; Beckton Dickinson, Hopkinton, MA) For gene expression experiments, nine rats received Ad gal-modified cells on one side and cells modified with AdNull on the other side. For fusion experiments, 10 rats received AdBMP-7-modified cells, 11 rats received AdNull-modified cells and 12 rats received unmodified cells bilaterally. As a "gold standard" control, 12 rats received fresh bone graft (50 mg/site) from syngeneic Lewis rats.
Duration of Study	3 & 6 weeks	3 & 6 weeks	Five rats each were killed 2, 4, 7, and 14 days after the surgery; Seven rats each were killed 14, 28, and 42 days after the surgery	8 weeks for fusion; 14 days for *in vivo* gene expression
Radiographs	3 & 6 weeks	3 & 6 weeks	At sacrifice: 2, 4, 7, and 14 days; At sacrifice: 14, 28, and 42 days	8 weeks

TABLE X2.1 *Continued*

Category	Publication Reference			
Radiographic Scoring	Blinded assessment by 2–3 reviewers, fusion determined if bridging bone was noted in either intertransverse region	Blinded assessment by 2–3 reviewers, fusion determined if bridging bone was noted in either intertransverse region	Fusion status of each specimen was graded based on three categories. The specimen was graded as a solid union when no motion was observed; as an immature union when bony continuity between L4 and L5 transverse processes was observed but the segment had slight motion; and as a nonunion when wide motion equivalent to adjacent segments was detected. Fusion rate was defined as the percentage of solid or immature union	Radiographs were evaluated by an expert observer blinded to the treatment groups. Samples were rated as fused if radiodense cortical bridging was present bilaterally. If discontinuities such as clefts or gaps were apparent, spines were graded as not fused, regardless of the presence of new bone formation.
Histology	Non-decalcified (toluidine blue stain)	Non-decalcified (Von Kossa stain)	Non-decalcified (toluidine blue O stain)	Non-decalcified (Goldner trichrome stain)
Histologic Scoring	Histologic fusion defined as bony trabeculae bridging from one transverse process to the next	Histologic fusion defined as bony trabeculae bridging from one transverse process to the next	None	None
Biomechanical Test Method	Manual palpation	Manual palpation	Manual palpation	Manual palpation; nondestructive flexion-extension testing under cyclic loading
Other Assessments	None	None	3D micro-CT: To evaluate the calcified fusion mass at the intertransverse process region where bone did not originally exist. The scans were initiated from the lower endplate of the L4 vertebral body cranially in 13-μm sections, for a total of 135 slices per scan. Histomorphometric analysis was performed to evaluate the fusion status and remodeling condition of the fusion mass. Mineral apposition rate (MAR) was calculated from the width of the double-labeled interval, and mineralized surface/bone surface (MS/BS) was calculated from the length of the bone surface and calcein-labeled surface. Osteoclast surface (Oc.S/BS) was calculated to evaluate bone resorption activity.	*in vivo* gene expression, histomorphometry (Bioquant Nova 2000)
Comments	Limitations: (*1*) Caution needed when extrapolating data to a higher animal models (that is, humans). (*2*) These models do not reflect the range of pathology or deleterious systemic agents in clinical situations.	Limitations: (*1*) Caution needed when extrapolating data to a higher animal models (that is, humans). (*2*) These models do not reflect the range of pathology or deleterious systemic agents in clinical situations. (*3*) Differences in loading environments between quadripedal animals and bipedal humans must be considered.	Limitations: Mechanical environment at the grafted segment and healing process of grafted bone are different than in humans. Study results are not fully translatable to human spinal arthrodesis surgery. No assessment of mechanical strength of the fusion segment.	Limitations: (*1*) Caution needed when extrapolating data to a higher animal models (that is, humans). (*2*) These models do not reflect the range of pathology or deleterious systemic agents in clinical situations.

TABLE X2.2 Published Examples for the Rabbit Posterolateral Spine Fusion Model

Category	Publication Reference					
Citation	Boden (55)	Kraiwattanapong (295)	Fredericks (296)	Singh (121)	Grauer (80)	Magit (97)
Breed	New Zealand white rabbit	New Zealand white rabbit	New Zealand white rabbit	New Zealand white rabbit	New Zealand white rabbit	New Zealand white rabbit
Chromosomal Sex	Male/female	Male/female	Male/female	Male/female	Female	Female
Age	1 year	1 year	None	1 year	"Adult"	1 year
Weight	4.5–5 kg	4.5–5 kg	4.5–5.5 kg	4.4–5.2 kg	4.5–5 kg	4.4 ± 0.3 kg
Group Size (n)	N=60 (10 per group)	N=24 (12 per group)	N=30 (15)	N=32 (16 per group)	N=31 (10, 12, 9)	N=67
Intertransverse Location	L5-L6	L5-L6	L4-L5	L5-L6	L5-L6	L5-L6
Control	Group 1 [N=2]: bone graft without decortication; Group 2 [N=2]: decortication without bone grafting	None	Autograft	Autograft	Autograft (positive control), carrier alone (negative control)	Autograft
Bone Graft Volume	2–2.5 cc	Group 1: 1.5 cc Healos + 1.5 cc BMA; Group 2: 1.5 cc rhBMP-2/ACS + 1.5 cc collagen-ceramic matrix (0.645 mg rhBMP-2 per side)	None	Group 1: 2.5 cc autograft; Group 2: 3.0 cc rhBMP-2/ ACS + autograft (0.43 mg rhBMP-2 per side)	1–1.5 cc of autograft per side; 0.3 g of bovine collagen I matrix and 77 mg of CMC per side; 0.3 g of bovine collagen I matrix and 77 mg of CMC + 1.2 mg OP-1 per side	Autograft: 1.5–2.0 cc per side; Healos (1.0 × 3.0 × 0.5 cm strip per side); Healos (1.0 × 3.0 × 0.5 cm strip per side) + 0.5 mg/cc rhGDF-5; Healos (1.0 × 3.0 × 0.5 cm strip per side) + 1.0 mg/cc rhGDF-5; Healos (1.0 × 3.0 × 0.5 cm strip per side) + 1.5 mg/cc rhGDF-5.
Bone Graft Material	Autograft	Group 1: 1.5 cc Healos + 1.5 cc BMA; Group 2: 1.5 cc rhBMP-2/ACS + 1.5 cc collagen-ceramic matrix (0.645 mg rhBMP-2 per side)	Autograft; autograft with bone stimulator	Group 1: 2.5 cc autograft plus IV doxorubicin; Group 2: 3.0 cc rhBMP-2/ACS + autograft (0.43 mg rhBMP-2 per side) plus IV doxorubicin	1–1.5 cc of autograft per side; 0.3 g of bovine collagen I matrix and 77 mg of CMC per side; 0.3 g of bovine collagen I matrix and 77 mg of CMC + 1.2 mg OP-1 per side	Autograft: 1.5–2.0 cc per side; Healos (1.0 × 3.0 × 0.5 cm strip per side); Healos (1.0 × 3.0 × 0.5 cm strip per side) + 0.5 mg/cc rhGDF-5; Healos (1.0 × 3.0 × 0.5 cm strip per side) + 1.0 mg/cc rhGDF-5; Healos (1.0 × 3.0 × 0.5 cm strip per side) + 1.5 mg/cc rhGDF-5.
Duration of Study	2, 3, 4, 5, 6, & 10 weeks	8 weeks	3, 7, 14, 21, 28 days	5 weeks	5 weeks	8 weeks
Radiographs	2, 3, 4, 5, 6, & 10 weeks	8 weeks	0 & 28 days	5 weeks	5 weeks	8 weeks
Radiographic Scoring	Blinded assessment, fusion determined as solid/not solid based on presence of continuous trabecular pattern within the intertransverse fusion mass	Blinded assessment, fusion determined as solid/not solid based on presence of continuous trabecular pattern within the intertransverse fusion mass	3 blinded assessments from independent reviewers, fusion determined as yes/no based on: (*1*) evidence of at least unilateral bridging fusion mass,(*2*) fully corticated fusion mass, (*3*) complete lack of bony cleft in fusion mass	5 blinded assessments from independent reviewers, bone formation graded via a 6-point scale listed in Table 1 in the publication	Blinded assessment, fusion determined as solid/not solid based on presence of continuous trabecular pattern within the intertransverse fusion mass	3 blinded assessments from independent reviewers, fusion determined based on presence of continuous trabecular pattern within the intertransverse fusion mass in either intertransverse region.