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

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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 hardware.

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, 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.*

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 Bone

F1983 Practice for Assessment of Compatibility of Absorbable/Resorbable Biomaterials for Implant Applications

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

2.2 *Other Standards*

ISO 10993 Biological Evaluation of Medical TEMPs—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 Product Standards – Sterility[4]

[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.

[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.

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 vertebra 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*—the vertebral column (singular: vertebra) are 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 (7 vertebrae), thoracic (12 vertebrae) and lumbar (5 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. 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 vertebra.

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 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 A 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 anount 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 hardware 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 regards 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 preclinical 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 hardware 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 12 weeks in life, long-term is 12 to 24 weeks or greater. In large animals (dogs, pigs, sheep, goats, non-human primates) short-term can be considered to mean less than 6 months in-life, and long-term 6 months or greater.

5.6.3 In small animals, small defects implanted for 5 to 12 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 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.

5.6.5 Periods of more than 3 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] The boldface numbers in parentheses refer to a list of references at the end of this standard.

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 6 to 8 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 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 grams (**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 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 alone 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 alone 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 alone 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 end-point 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 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 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 the appendix..

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/ non-mineralized) 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 6 months for large animals and less than 12 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 non-destructive 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 non-destructive 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 week 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	2cc/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	rRadiographs 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 trichromestain)
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; non-destructive flexion-extension testing under cyclic loading
Other Assessments	None	None	3-D 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.

TABLE X2.2 *Continued*

Category	Publication Reference					
Histology	N=2 at each timepoint; non-decalcified (hematoxylin and eosin OR Goldner Trichrome)	N=3 at from each group; non-decalcified (1% methylene blue and 0.3% basic fuchsin)	None	None	all specimens; decalcified (hematoxylin and eosin) & non-decalcified (toluidine blue)	all specimens; decalcified (hematoxylin and eosin) & non-decalcified (Von Kossa toluidine blue)
Histologic Scoring	Qualitative assessment performed, but no grading scale used	Qualitative assessment performed, but no grading scale used	None	None	Qualitative assessment performed, but no grading scale used	3 blinded assessments from independent reviewers graded the hematoxylin and eosin stained sections on a measuring scale of 1-10, listed in Table 1 of the publication. Fusion was defined as a score >6, representing the appearance of continuous bridging of bony trabelculae across adjacent transverse processes.
Biomechanical Test Method	Manual palpation; uniaxial tensile loading (ultimate tensile load, stiffness) normalized to adjacent unfused level	Manual palpation; uniaxial tensile loading (ultimate tensile load, stiffness) normalized to adjacent unfused level	None	Manual palpation and graded according to size and on a five-tiered classification scale listed in Table 1 in the publication.	Manual palpation - 2 blinded assessments, fusion determined as solid/not solid based on no significant motion present; multi-directional ROM flexibility testing using Optotrak motion system	Manual palpation - 3 blinded assessments, fusion determined as solid/not solid based on no significant motion present
Other Assessments	None	CT scans	None	None		CT scans of representative specimens
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. (*3*) Biomechanical data on fusions was limited.	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.	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.	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 X2.3 Published Examples for the Dog Posterolateral Spine Fusion Model

Category	Publication Reference					
Citation	Asher (144)	Cunningham (145)	Delcrin (146)	Farey (147)	Jarzem (150)	Sandhu (152)
Breed	Unspecified Canine	Skeletally mature purpose-bred coonhounds	Adult beagle dogs	Adult beagle dogs	Mature mongrel dogs	Adult beagle dogs
Chromosomal Sex	Male	Not specified	Not specified	Not specified	Not specified	Female
Age	2.4 ± 1.8 yr	2-3 years	Adult	Adult	Adult	Adult
Weight	36 ± 5.1 kg	20 kg	11-13 kg	Not specified	~ 20 kg	Not specified
Group Size (n)	N=26 (2-4 per group)	N=24/treatment for total of 72 fusion sites (6 per group)	N=13	N=7	N=13	Group 1: N=6, rhBMP-2-OPLA composite; Group 2: N=6, autograft; Group 3: N=2, OPLA only
Intertransverse Location	L3-L5	L3-L4, L5-L6	L2-L4	L5-L6	L5-L6	L4-L5
Control	Group 1 [N=4, 6mo., N=2, 12 mo.]: Sham, Screws Removed, Grafts Discarded;	Group 1 (Autograft);	Group 3 (autograft)	Group I (n = 7), destabilized, animals followed up 6 months after anterior retroperitoneal LSL6 diskectomy, resection of the anterior longitudinal ligament at L5-L6, and posterior L5-L6 laminectomy and facetectomy, no fusion and no instrumentation	allograft	autograft
Bone Graft Volume	Not Quantified	Autograft (4 g total, 2 g/side); Autograft + OP-1 (1 g autograft + 1 g OP-1 Putty); OP-1 Putty alone (2 g total, 1g/side)	Autograft amount not quantified; ceramic used was a macroporous biphasic material composed of 40% [beta] TCP and 60% HA (Triosite, Zimmer, France) shaped into 20 mm × 5 mm × 5 mm parallelepipedic blocks	1.5 cm^2	Control: 15 cm^3 of allograft/side ; Experimental: 15 cm^3 of allograft/side + 1 cm^3 of fibrin adhesive (Tisseel)	Autograft: 2.2 cc/side; 12 mm × 6 mm × 30 mm strips for OPLA; 1 cc rhBMP-2 solution combined with OPLA strips
Bone Graft Material	Group 2 [4.76 mm Rod; N=4, 6 mo. & 12 mo.]: Facet, posterolateral, and posterior arthrodesis (N=4, 6 mo.; N=4, 12 mo.); Group 3 [6.35 mm Rod; N=4, 6 mo. & 12 mo.]: Facet, posterolateral, and posterior arthrodesis (N=4, 6 mo.; N=4, 12 mo.);	Group 2 (OP-1 Putty + Autograft); Group 3 (OP-1 Putty alone)	Group 1: N=4, 3 ceramic blocks aligned in both laterovertebral grooves and 2 blocks in the area of the transverse processes; Group 2: N=4, ceramic blocks only in both laterovertebral grooves; Group 3: N=5 cancellous autogenous bone graft harvested from the posterior iliac crest and placed on the laminar and intertransverse sites	Iliac crest autograft	Control: 15 cm^3 of allograft/side ; Experimental: 15 cm^3 of allograft/side + 1 cm^3 of fibrin adhesive (Tisseel)	Autograft; OPLA; rhBMP-2 + OPLA
Duration of Study	6 mo, 12 mo	4, 8, 12, 24 weeks	9 mo	6 mo	26 weeks	3 mo, 8 mo

TABLE X2.3 *Continued*

Category			Publication Reference			
Radiographs	None	4, 8, 12, 24 weeks	None	Microradiographs were made using cross sections 25 μm in thickness	CT (axial 4mm cuts at 4 mm intervals)	CT (2, 3, & 8 mo), Faxitron (sacrifice)
Radiographic Scoring	None	Grading scale 1. A: Solid bilateral fusion: clearly solid transverse process fusion bilaterally with confluent trabeculated bone extending from transverse process to transverse process. 2. B: Solid unilateral fusion: clearly solid transverse process fusion unilaterally with confluent trabeculated bone extending unilaterally from transverse process to transverse process. 3. C: Partial union: evidence of bone growth between the transverse processes either unilaterally or bilaterally, but with lucency indicative of nonconfluent trabeculation. 4. D: Nonunion: no evidence of bone growth between the transverse processes.	None	None	Volumetric fusion assessment (greater than 1.2 cm^3)	CT scale: bilateral bridging w/ isodense bone; bilateral bridging w/ hypodense bone; Unilateral bridging; Incomplete bridging; No new bone Faxitron scale: Complete bilateral osseous bridging; Complete unilateral osseous bridging; Incomplete bridging; No new bone formation
Histology	None	Non-decalcified histology, stained with Villanueva Osteochrome Bone Stain; High-resolution microradiographs permitted histomorphometric quantification of posterolateral trabecular bone areas (mm^2); Decalcified histology using hematoxylin and eosin stain; plain and polarized light microscopy, histopathological assessment for all tissues included, but was not limited to, comments on trabecular architecture, presence of collagen, as well as any signs of foreign body giant cell/granulomas inflammatory reactions, degenerative changes or autolysis. Moreover, the developmental ossification process of new bone, intramembranous or endochondral, was evaluated in all treatment groups for each postoperative time period.	Non-decalcified, tained with solochrome cyanine R	Light microscopy was performed on non-decalcified sections stained with hematoxylin and eosin. Toluidine blue was used to help differentiate mineralized from unmineralized osteoid. Non-decalcified sections 10 μm in thickness were evaluated under fluorescent light for tetracycline, alizarin, DCAF, and xylenol orange uptake.	None	Undelcalcified & decalcified, toluidine blue-O & basic fuchsin stains.

TABLE X2.3 *Continued*

Category	Publication Reference					
Histologic Scoring	None	None; Histomorphometric data represents the area (mm^2) of trabecular bone formation within the intertransverse space	To evaluate bone in-growth as a function of the local environment, transverse sections of each implanted block from both sites were divided into nine square areas, which were considered separately and by groups of three squares to obtain three groups: dorsal squares, no. 1 + no. 2 + no. 3; middle squares, no. 4 + no. 5 + no. 6; and ventral squares, no. 7 + no. 8 + no. 9. The relative area of newly formed bone inside the pores and the total pore area, which was considered to be the sum of the bone in-growth area and the area of empty space, were measured in relation to the total implant area and expressed as a percentage for each of the nine different areas. The total pore area indicated the changes in porosity. Measurements of non-decalcified transverse sections on microradiographs were obtained semiautomatically using a computer-assisted image analyzer. Light microscopic manual analysis on stained sections was used to validate the semiautomatic image analysis on microradiographs.	Quantitative histologic analysis of bone was performed using the semiautomatic method of Malluche et al.	None	
Biomechanical Test Method	Axial flexion-compression stiffness of the L3-L5 segment components and axial compression stiffness of the bypassed and adjacent anterior column elements were measured.	Peak range of motion for each loading mode was calculated as the sum of motions (maximum displacement [millimetres] for axial compression or maximum rotation for torsion, flexion-extension and lateral bending [degrees]) occurring in the neutral and elastic zones at the fourth loading cycle.	Four-point bending apparatus was designed to provide a loading mode representing a pure bending moment; flexion, extension, and lateral bending	None	Force-displacement measurements of fusion mass done using customized jig (fusion assessed as stiffness less than or equal to 1.07 mm/N).	Pure torques and linear loads in flexion-extension, lateral bending, and rotation.
Other Assessments	None	None	None	None	None	None

TABLE X2.3 *Continued*

Category	Publication Reference					
Comments	Limitations: (*1*) Caution needed when extrapolating data to higher animal models (that is, humans). (*2*) These models do not reflect the range of pathology or deleterious systemic agents in clinical situations. (*3*) Did not address adjacent vertebra; (*4*) Small numbers of canines in each group; (*5*) Differences in loading environment between quadripedal animals and bipedal humans must be considered. (*6*) same canine could not be used for control tests followed by sham or instrumentation and arthrodesis	Limitations: (*1*) Caution needed when extrapolating data to 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 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 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 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 higher animal models (that is, humans). (*2*) These models do not reflect the range of pathology or deleterious systemic agents in clinical situations.

TABLE X2.4 Published Examples for the Dog Interbody Spine Fusion Model

Category	Publication Reference					
Citation	Ohyama (160)	McAfee (158)	Emery (157)	Shima(161)	Shirado(162)	Cook (155)
Breed	Colony-reared adult dogs	Beagle	Beagle	Mongrel dog	Coon hound	Colony-bred hounds
Chromosomal Sex	Male	Not specified	Female	Not specified	Not specified	Male
Age	1-9 mo	1 yr	~ 1 yr	Adult	Adult	2 yr
Weight	17-18 kg	Not specified	8.5-10.5 kg	11-20 kg	20-25 kg	25-30 kg
Group Size (n)	N=8 (3 disc spaces per dog)	N=6/group	N=20	N=20 (2 sites/animal; 5 groups, 4/group)	N=21 (7/Group)	N=21
Interbody Location	L1-L2, L2-L3, L3-L4, L4-L5	L5-L6	T7-T8	C3-C4, C5-C6	L4-L6	C3-C4, C5-C6
Control	Group A: autograft;	Group 1: surgically destabilized, no graft	Tricortical iliac crest graft	Autologous humerus graft	Group 1: strut bone graft alone	Autogoenous corticocancellous tri-cortical graft
Bone Graft Volume	Autograft not quantified; 0.3g of beta-TCP granules for Groups B & C; 0.2 mL of rhBMP-2 solution in Group C.	Not specified	Not specified	Not specified	None	10 mm × 10 mm autogenous tri-cortical graft; HA implants 14 mm × 12 m × 5 mm.
Bone Graft Material	Group B: beta-TCP; Group C: rhBMP-2 + beta-TCP	Group 2: autograft; Group 3: autograft with fibular strut graft	Group I: Autogenous iliac crest bone graft. (n = 6). Group II: Hydroxyapatite ceramic (HA; Interpore, Irvine, CA, n = 6). The pore size is 200 μm. Group III: Biphasic (60: 40) hydroxyapatite/tricalcium phosphate ceramic (HA/TCP; Zimmer, Warsaw, IN, n = 4), with an average pore size of 400 μm. Group IV: Calcium carbonate ceramic (CC; Inoteb, Le Guernol, France, n = 4). We used the 20% porosity product, which has an average pore size of 250 μm. All ceramic cubes had been machined to a 6 × 6 × 6 mm size preoperatively for a congruent fit.	Autologous humerus graft; TCP dowel (Synthos)	Ulna strut graft; ulna strut graft w/ polyanhydride copolymer (4 cm length × 1 cm DIA)	autogenous tri-cortical graft; Hydroxyapatite implant (Calcitek)
Duration of Study	16 weeks	6 mo	8 weeks	3, 6, 12, 18, 22 weeks	6 mo	6, 12, & 26 weeks
Radiographs	Lateral & AP (post-op, 2, 4, 8, 12, & 16 weeks)	6 mo	After biomechanical testing, AP and lateral radiographs were obtained	3, 6, 12, 18, 22 weeks at sacrifice	Microradiographs	Plain radiographs post-op; CT 3 mm thick and 3 mm increments

TABLE X2.4 *Continued*

Category	Publication Reference					
Radiographic Scoring	High resolution microradiographs using a computerized histomorphometric system was used to evaluate fusion status	None	Qualitiative assessment only (fused/not fused – one reviewer)	Fusion criteria and scoring not specified	None	None, qualitative
Histology	Non-decalcified, stained using toluidine blue	None	Non-decalcified, toluidine blue stain; Unstained, fluorochrome analysis	Decalcified & Non-decalcified, H&E and masson's trichrome stain	Non-decalcified, hematoxylin, eosin, and toluidin blue	Non-decalcified, basic fuchsin and toluidine blue
Histologic Scoring	None	None	Qualitative assessment only	Qualitative assessment only	Volumetric density of bone and mean trabecular diameter of bone calculated	% bone apposition to graft materials
Biomechanical Test Method	Pure torques and linear loads in flexion-extension, lateral bending, and rotation.	Torsional and axial compressive stiffness	Non-destructive testing using axial compression and displacement loading for flexion-extension, torsion, and lateral bending;	None	Non-destructive biomechanical testing	Rotational torque non-destructive testing ; flexion-extension destructive testing using modified 4-point bending configuration
Other Assessments	None	None	None	Two-color fluorescent labeling (Suzuki & Matthews) used to determine state of osteogenesis at time of operation and sacrifice.	None	None
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.	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.	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. (*4*) Mathematical assumptions on histomorphometric analysis.	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 X2.5 Published Examples for the Sheep Posterolateral Spine Fusion Model

Category	Publication Reference					
Citation	Wheeler(180)	Gupta(168)	Walsh (178)	Kanayama (171)	Baramki(166)	Kim (173)
Breed	Sheep	Cross sheep	Cross sheep	Suffolk Sheep	Dorset sheep	Sheep
Chromosomal Sex	Female	Female	Not specified	Not specified	Female	Female
Age	Skeletally mature	3-4 years	Not specified	Skeletally mature	Skeletally mature	Skeletally mature
Weight	Not specified	~ 150 lb	45-55 kg	Not specified	Not specified	50-75 kg
Group Size (n)	N=18 (9/group)	N=24 (6/group)	N=24 (8/group)	N=16 (8/group)	N=28 (7/group)	N=12
Posterolateral Location	L4-L5	L4-L5	L3-L4	L2-L3, L4-L5	L3-L4, L4-L5	L2-L3, L5-L6
Control	N=9, iliac crest autograft	Iliac crest autograft	Iliac crest autograft corticocancellous bone strips	Iliac crest autograft and local bone	Group 2: iliac crest autograft	Iliac crest autograft
Bone Graft Volume	20 cc (10 cc/side)	10 cc (5 cc/side)	Two strips of either corticocancellous bone graft (5 mm × 50 mm), Collagraft strips (5 mm × 50 mm) soaked in saline, or Collagraft strips soaked in saline then coated with bone marrow aspirated from the iliac crest were used for each side of the fusion site.	20 g/segment	Group 2: 30 cc iliac crest autograft; Group 3: 30 cc IPH (ProOsteon 500); Group 4: 15 cc local bone and 15 cc IPH.	Autograft: 10 cc (5 cc/side); 20 cc (10 cc/side)
Bone Graft Material	Iliac crest autograft; Si-CaP (Actifuse Synthetic Bone Graft (ApaTech Limited, London)	Group 1: Iliac crest autograft; Group 2: SCR-enriched TCP (Conduit, DePuy Spine); Group 3: TCP soaked with whole bone marrow; Group 4: TCP alone	Iliac crest corticocancellous bone strips; Collagraft hydrated with saline; Collagraft hydrated with saline plus bone marrow	Autograft and local bone	Group 2: iliac crest autograft; Group 3: IPH (ProOsteon 500); 15 cc local bone and 15 cc IPH.	Iliac crest autograft; HealosMP52
Duration of Study	180 days	6 mo	6 mo	8 weeks, 16 weeks	20 weeks	6 mo, 12 mo
Radiographs	CT scans at 60, 120, & 180 days for Si-CaP group; CT scans at 60 & 180 days for autograft group	Plain radiographs post-op and necropsy, micro-CT	AP radiographs at 2, 4 & 6 mo;	Plain radiographs, CT, microradiography	Plain radiographs, CT	AP plain radiographs (2 blinded reviewers); CT

TABLE X2.5 *Continued*

Category	Publication Reference					
Radiographic Scoring	Bilateral qualitative fusion scores (0=no fusion, 1=moderate fusion and thin connectivity, 2=extensive fusion and connectivity) were made based on rendered 3D image by a single qualified investigator blinded to the treatment. The density of the fusion mass was categorized based on the density phantom into four categories: 400–600, 600–800, 800–1000, 1000–1250 mg/cc. The percentage of the total fusion volume at each density range was calculated to quantify densification of the tissue. One animal received a CT scan of the Si-CaP graft material immediately after implantation to characterize initial graft volume and density. To measure graft resorption and new bone formation, specific image slices within the 3D image stack were evaluated at each time point. Changes in fusion mass density based on shifts in the four density categories and fusion volume were calculated and provided information to estimate Si-CaP graft resorption and bone formation over time; Routine ventrodorsal and lateral radiographs of the explanted spinal segments were performed. Radiographs were qualitatively scored, blinded to treatment group, based on the extent of right and left fusion mass and connectivity using the same scale applied for the CT scans (0,1,2)	CT: Each spine segment was rated using Sandhu's fusion rating scale: 0 = no fusion; 1= bone formation, but no fusion; 2 = unilateral fusion; 3 = bilateral fusion	Qualitative assessment only	Qualitative assessment by three blinded reviewers – solid union was defined as complete and contiguous bridging of the transverse processes	CT (2 independent radiologists): 4-point grading system to determine bone bridging and resorption. Grade 1: 76% to 100% bridging within the graft; Grade 2: 51% to 75% bridging within the graft; Grade 3: 26% to 50% bridging within the graft; Grade 4: 0% to 25% bridging within the graft; Grade 1 resorption: radiolucency was 0% to 25% of the graft area; Grade 2 resorption: radiolucency was 26% to 50% of the graft area; Grade 3 resorption: radiolucency was 51% to 75% of the graft area; Grade 4 resorption: radiolucency was 76% to 100% of the graft area	Grading Scale: 0 = no bony response; 1 = bony response but incomplete bridging; 2 = complete bridging, but less than half width of adjacent transverse process height in the mid-intertransverse space; 3 = complete bridging, but less than full width of adjacent transverse process height in the mid-intertransverse space; 4 = complete bridging, with greater than full width of adjacent transverse process height in the mid-intertransverse space; Maximum grade for each fusion site and graft material was 8.
Histology	Non-decalcified (Von Gieson stain)	Non-decalcified (toluidine blue stain)	Non-decalcified, UV labeled evaluation; hematoxylin and eosin, von Kossa, and toluidine blue stains; plain radiographs taken of the thick histologic sections prior to polishing to examine radiographic appearance	Non-decalcified (Osteochrome Villanueva bone stain)	None	Non-decalcified (Sanderson rapid Bone Stain and counterstained with Acid Fuchsin) Decalcified (osteosarcoma analysis, hemotoxylin and eosin (H&E) stain)

TABLE X2.5 *Continued*

Category	Publication Reference					
Histologic Scoring	Healing, bone quality, and graft incorporation were scored using a semiquantitative scale. The scale graded graft–tissue interface (0=gap, 1=fibrous, 2=fibrous and bone, 3=bone), remodeling (0=woven, 1=woven>lamellar, 2=woven<lamellar, 3=lamellar), osteoblasts (0=minimal, 1=some, 2=many), osteoclasts (0=minimal, 1=some, 2=many), and inflammation (0=None, 1=some, 2=many). Scores were assigned by a single pathologist in 0.5 increments.	The purpose of the histology was to assess: (*1*) the overall morphology of *de novo* bone; (*2*) the maturity of the bone and presence of osteoid and active osteoblasts and osteoclasts; (*3*) the amount of residual implant material; and (*4*) the presence of any other cell type in the fusion site.	Qualitative assessment only	None	Qualitative assessment only	Qualitative assessment only
Biomechanical Test Method	None	Manual palpation	Right & left 3-point bending applying non-destructive load up to 100 N at 2mm/min; Destructive testing in flexion-extension at 50 mm/min; The peak load, energy to peak load and stiffness, and mode of failure were determined.	Manual Palpation (graded as solid/not solid); nondestructive testing under load control in compression, flexion-extension, lateral bending, and axial rotation.	Nondestructive pure moment testing (6 DOF)	Manual palpation (2 blinded reviewers, solid/not solid)
Other Assessments	Histomorphometry; Histomorphometric parameters measured or calculated included: total reactive (area of the fusion) area (mm^2), bone within reactive area (mm^2), percent bone within reactive area (%), graft within reactive area (mm^2), percent graft within reactive area (%), percent graft+bone within the reactive area (%), distance between transverse processes (mm), connecting bone within the transverse process span (mm), and percent distance between transverse processes consisting of bone (bone union) (%).	Micro-CT	None	Histomophometry, assessed total fusion mass area, trabecular bone area, and relative trabecular bone area.	None	Backscattered electron image analysis (to assess bone volume); High resolution contact radiographs (total volume fraction of bone present)

TABLE X2.5 *Continued*

Category	Publication Reference					
Comments	Pedicle screw stabilization used; Limitations: (*1*) Caution needed when extrapolating data to 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 higher animal models (that is, humans). (*2*) These models do not reflect the range of pathology or deleterious systemic agents in clinical situations.	Pedicle screw stabilization used; Limitations: (*1*) Caution needed when extrapolating data to higher animal models (that is, humans). (*2*) These models do not reflect the range of pathology or deleterious systemic agents in clinical situations.	Pedicle screw stabilization used and demonstrated faster healing of fusion; Limitations: (*1*) Caution needed when extrapolating data to higher animal models (that is, humans). (*2*) These models do not reflect the range of pathology or deleterious systemic agents in clinical situations.	Pedicle screw stabilization used; Limitations: (*1*) Caution needed when extrapolating data to higher animal models (that is, humans).(*2*) These models do not reflect the range of pathology or deleterious systemic agents in clinical situations.	Pedicle screw stabilization used; Limitations: (*1*) Caution needed when extrapolating data to higher animal models (that is, humans).(*2*) These models do not reflect the range of pathology or deleterious systemic agents in clinical situations.

TABLE X2.6 Published Examples for the Sheep Interbody Spine Fusion Model

Category	Publication Reference					
Citation	Sandhu (201)	Ito (192)	Slivka (203)	Kandziora (193)	Takahata (205)	Thomas (213)
Breed	Merino sheep	Suffolk sheep	Rambouillet X Columbia sheep	Merino Sheep	Suffolk sheep	Rambouillet X Columbia sheep
Chromosomal Sex	Female	Male	Female	Female	Not specified	Female
Age	Skeletally mature	1-2 years	>3.5 years	2 years	2-3 years	Skeletally mature
Weight	~ 65 kg	65-80 kg	Not specified	Not specified	65-80 kg	Not specified
Group Size (n)	N=12 (6/group)	N=21 (7/group)	N=15 (2 sites/animal; 6 sites/group)	N=24 (8/group)	N=24 (2 levels per animal)	N=8
Interbody Location	L4-L5	L2-L3, L4-L5	C2-C3, C4-C5	C3-C4	L2-L3, L4-L5	C3-C4, C4-C5, C5-C6
Control	Autograft	None	CFRP cage alone	Autologous tricortical iliac crest bone graft	None	None
Bone Graft Volume	Autograft: not specified; rhBMP-2: one 1" × 2" sponge	None	Not Specified	Group 2 & 3: 0.4 cc iliac crest autograft	Not specified	Not specified
Bone Graft Material	Autograft; rhBMP-2 on ACS	None, HAC spacer w/ varying porosity only	Autograft harvested from the sternum	Iliac crest autograft	Smooth and porous surface ceramic blocks (23 mm × 13 mm × 10 mm)	Autograft harvested from the sternum
Duration of Study	6 mo	4 mo, 6 mo	6 mo	12 weeks	2, 4, 8, 12, 24, 52 weeks	6, 12, & 36 mo
Radiographs	Post-op, 2, 4, & 6 mo	CT (at sacrifice)	Dorsal-ventral & lateral @ 3 & 6 mo.	Dorsal-ventral & lateral plain film (1, 2, 4, 8, 12 weeks); CT	AP and lateral plain radiographs (harvested spines);	Dorsal-ventral and lateral radiographs were made before and after removal of the metallic plates and graded to assess fusion.
Radiographic Scoring	A score was assigned depending on absence (Grade 0) or presence of bone anterior to the cage. When bone was present and there was an attempt at bridging from one side only, that sample scored Grade 1. When there was "attempted" bridging from both sides, the sample was assigned Grade 2, and Grade 3 was assigned if the attempted fusions from the cephalad and caudad ends of the vertebrae were one continuous mass but this mass did not project beyond the anterior margin of the vertebral bodies. When the fusion mass extended in front of the anterior margins of the contiguous vertebrae, this was assigned a Grade 4. The observer (A.D.D.) was blinded to the groups when scoring the radiographs. Radiographs were available for five animals in the autograft group.	Bonding conditions between HAC and vertebral body analyzed and classified into 4 grades: protrusion, suspicious fusion, probable fusion, absolute fusion.	Each treatment site was judged by the primary author (M.A.S.) as (*1*) not fused, with a clear radiolucency present across the disc space; (*2*) partly fused, characterized by evidence of bridging, mineralized callus across the disc space; or (*3*) fused, clearly showing a solid bridge of bone spanning the disc space.	Plain films: At 1, 2, 4, 8, 12 weeks, anterior, middle, and posterior intervertebral disc space heights (DSH) of the motion segment C3/C4 were measured on lateral radiographic scans. Average intervertebral DSH was calculated from anterior, middle, and posterior DSH measurements (anterior _ middle _ posterior DSH/3). Functional radiographic assessment using lateral radiographs post-sacrifice. (Three independent reviewers); CT: BMD and bony callus measurement	Radiographic assessment was performed independently by three orthopedic surgeons who were blinded with regard to the mechanical and histologic data, and the radiographic fusion rate was calculated at each time-period by averaging the results of the three observers.	The Faxitron radiographs were graded as follows: 1 _ nonfusion; 2 _ lucency with some bony bridging; 3 _ increased bone density; 4 _ continuous bony bridging.

TABLE X2.6 *Continued*

Category	Publication Reference					
Histology	Non-decalcified (toluidine-blue-O and basic fuchsin stain)	4 motion segments from each group analyzed; Non-decalcified (H&E and toluidine-blue-O stain)	None	Non-decalcified; Stains used: (*1*) safranin-O/ Lightgreen, (*2*) safranin-O/ van Kossa, (*3*) astrablue, and (*4*) Masson-Goldner. Masson-Goldner stainings were used for histomorphological analysis	Non-decalcified (toluidine-blue-O stain for vertebral interface, H&E for facet joint)	Non-decalcified (trichrome stain)

TABLE X2.6 *Continued*

Category						
	Publication Reference					
Histologic Scoring	The nature of the fusion was commented on after a detailed analysis of 10 randomly selected un-decalcified stained sagittal sections of the fusion level for separate animals. Evaluation included qualitative assessment of osteogenesis in contact with the TEMPs and in the open pores of the titanium TEMPs as well as the histologic and cytologic host response in the vicinity of the titanium TEMPs. In each sagittal section the presence of intervertebral fusion anterior to, posterior to, or through the TEMPs was determined as follows: (*1*) an uninterrupted bridge of bone present in the anterior margin was considered an anterior fusion, (*2*) an uninterrupted bridge of bone present in the posterior margin was considered posterior fusion, or (*3*) continuous bone ingrowth from the endplate of the cephalad vertebrae through the superior, middle, and inferior portions of the TEMPs and into the endplate of the caudal vertebrae was considered fusion through the TEMPs. Based on these criteria, each specimen was assigned an overall rating of fusion as follows: (*1*) complete fusion, (*2*) partial or incomplete fusion, or (*3*) nonfusion. A rating of complete fusion was given if the majority of sections of a specimen depicted a complete intervertebral bridge of bone. If intervertebral bridging was present either through the metallic TEMPs or anterior or posterior to it but was noted in less than a majority of sections, then a rating of partial fusion was given. If no sections depicted a complete, uninterrupted, intervertebral bridge of bone, then the sample was rated as nonfusion. After the analysis by the orthopedic pathologist, sections for each sample that were representative of the fusion status were selected, photographed, and further analyzed in a blinded fashion by one of the investigators (A.D.D.). To study the continuity of bone inside the cage (intracompartmental) with that outside the cage (extracompartmental), bony continuity was evaluated at the fenestrations of the cage in sagittal sections. Specifically, a trapezoidal template was drawn centering on each fenestration of the titanium cage and to include the contiguous intracompartment and extracompartment regions measuring half the thickness of the cage from the respective inner or outer rim of the cage. A fenestration was labeled “all bone” if only bone was observed (blue stained tissue), “partial bone” if there was a mixture of bone and fibrous tissue (pink stained tissue), or “no bone” if only fibrous tissue was seen. Results were expressed as a percentage for each sample.	Direct bonding of the HAC spacer was quantified using 3 slices from one HAC spacer and determined using ratio of the direct bonding surface to the total surface of HAC.	None	Intervertebral fusion was categorized histologically according to Zdeblick. The bone graft or cage-bone interface and the tissue content inside the cages were analyzed (1. cage/ vertebral interface: empty = 0 points; fibrous tissue = 1 point; bone = 2 points; 2. tissue inside cage: empty = 0 points; fibrous cartilage = 1 point; bone = 2 points). If four points were awarded, a successful arthrodesis or fusion was considered to have occurred. Foreign body reactions associated with the bioabsorbable implants were graded histologically according to the score of Hoffmann. 0 None No osteolysis 1 Mild Osteolysis around the implant< 1 mm (osteolysis zone) 2 Moderate Osteolysis around the implant 1–3 mm (cystic osteolysis) 3 Severe Osteolysis around the implant >3 mm (confluent osteolysis) 4 Extensive osteolysis around the implant >3 mm plus implant breakdown	Qualitative assessment only	Based on all sections evaluated from each spine, the spinal level was considered to be fused if greater than 50 % of the sections showed continuous bony bridging in any of the 3 anatomic regions; a partial fusion existed if less than 50 % of the sections showed continuous bony bridging in any of the 3 anatomic regions, and a nonfusion existed if none of the sections showed continuous bony bridging.

TABLE X2.6 *Continued*

Category	Publication Reference					
Biomechanical Test Method	Nondestructive testing using pure bending moments that were applied to the motion segments L3-L6 to induce flexion, extension, left and right lateral bending, and left and right axial rotation; 14 unoperated cadaveric sheep spines were tested as controls. One observer in a blinded fashion evaluated the amount of fibrous tissue in and around the cage as an index of poor fusion (more fibrous tissue = poorer histologic fusion). A rectangular grid was overlaid around the cage that was tangential and touching the caudad and cephalad outer margins of the cage and extending two cage thicknesses outside the anterior and posterior margins. Percentage areas representing the cage, fibrous tissue (pink), and bony tissue (blue–purple) were estimated for each representative section in both groups of animals.	Destructive tensile testing under displacement control.	Nondestructive testing using pure bending moments that were applied to the motion segments C3/C4 to induce flexion, extension, left and right lateral bending, and left and right axial rotation; each treatment level was graded as fused if the average ROM for each of the three test modes was less than 4°; otherwise, it was graded as not fused, as previously reported by Cunningham, et al.	Nondestructive testing using Pure bending moments that were applied to the motion segments C3/C4 to induce flexion, extension, left and right lateral bending, and left and right axial rotation.	Non-destructive testing using axial compression and pure moment loading for flexion-extension and lateral bending; indirect assessment of the stiffness of the anterior spinal fusion mass by measuring rod strain of the spinal instrumentation using uniaxial strain-gauges;	Nondestructive testing using Pure bending moments that were applied to the motion segments C3/C4 induce flexion, extension, left and right lateral bending, and left and right axial rotation;
Other Assessments	Microradiographs	Micro-CT	Micro-CT to assess new bone formation	Histomorphometry	Micro-CT (qualitative assessment to assess fusion mass)	Microradiographs
Comments	Limitations: (*1*) Caution needed when extrapolating data to 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 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 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 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 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: (*1*) Caution needed when extrapolating data to 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. (*4*) The number of animals used in the study was low. (*5*) Time points for histological assessment were limited.

TABLE X2.7 Published Examples for the Goat Interbody Spine Fusion Model

Category	Publication Reference					
Citation	Lippman(230)	Mooney(231)	Krijnen(227)	Brantigan(223)	Takahashi(239)	Pintar(234)
Breed	Goat	Goat	Dutch milk goats	Spanish goat	Goats	Goats
Chromosomal Sex	Male	Male	Female	Not specified	Female	Not specified
Age	1-2 years	Adult	Skeletally mature	1-2 years	Not specified	Not specified
Weight	Not specified	Not specified	45-70 kg	23-32 kg	40-55 kg	30-40 kg
Group Size (n)	N=42 (N=8 1 cm × 2 cm tricortical iliac bone autograft, N=16 70/30 PLDLLA/PGA + autograft or rhBMP-2, N=18 85:15 PLDLLA/PGA + autograft or rhBMP-2)	N=9 (3/group)	N=35 (28 stand alone [14 titanium, 14 PLDLLA], 7 PLDLLA w/ anterior fixation)	N=27 (17 iliac crest, 10 allograft)	N=14 (3 disc spaces/animal; 2 animals received only control grafts at all 3 levels)	N=14 (56 spinal units, 4/animal)
Interbody Location	C2-C3, C3-C4, C4-C5	L4, L5, L6 (actually placed into vertebral body)	L3-L4	L4-L5	C3-C4, C4-C5, C5-C6	C2-C3 or C3-C4 and C4-C5 or C5-C6; 2 lumbar levels implanted (not specified), with an intact space between.
Control	1 cm × 2 cm tricortical iliac bone autograft	Autograft	Titanium implant w/ autograft	Allograft	Porous HA graft w/ PBS	tricortical iliac bone autograft in one lumbar site and one cervical site
Bone Graft Volume	1 cm × 2 cm tricortical iliac bone autograft; 2 cc of iliac crest autograft for the PLDLLA/PGA cages	Not specified	Not specified	Not specified	15 mm × 15 mm × 8 mm graft	Not specified
Bone Graft Material	Tricortical iliac bone autograft, 70/30 PLDLLA/ PGA + autograft or rhBMP-2, 85:15 PLDLLA/PGA + autograft or rhBMP-2	Autograft; HA granules (dense and porous)	Iliac crest autograft	Allograft; iliac crest autograft	Porous HA graft w/ PBS; Porous HA graft w/ 5 µg of rhBMP-2; Porous HA graft w/ 50g of rhBMP-2	Tricortical iliac bone autograft; HA (Calcitek) blocks (14 mm × 12 mm × 6, 8, or 10 mm)
Duration of Study	3 mo, 6 mo, 12 mo	3 mo	PLDLLA stand alone: 3 (N=6), 6 (N=7), and 12 mo (N=8); PLDLLA fixation 6 mo; titanium 12 mo.	6, 12, 24 mo	4 weeks, 12 weeks	6 weeks (N=4), 12 weeks (N=4), 24 weeks (N=6)
Radiographs	1, 6, 12, 24 weeks	Standard radiographs were performed in all animals to document placement of fusion cages.	MRI at sacrifice (to assess cage integrity); Lateral radiographs of sections used to evaluate fusion	Lateral (post-op); plain film and 3-D CT at sacrifice	Immediately post-sacrifice, evaluated by two blinded evaluators; CT scans; DEXA scans	AP and lateral plain films post-op, 1 week, 2 weeks, 3 weeks, 5 weeks, 7 weeks, 9 weeks, 11 weeks, 13 weeks, 15 weeks, 17 weeks, 19 weeks, 21 weeks, 23 weeks, sacrifice; CT done after explant

TABLE X2.7 *Continued*

Category	Publication Reference					
Radiographic Scoring	Radiographic scoring (single reviewer): 0 no intervertebral or transvertebral osseous densities; 1 fragmented intervertebral or transvertebral osseous densities; 2 transvertebral osseous bridge	None	3-point scoring system: 0 = no ingrowth of bone into cage; 1 = ingrowth of bone with the cage securely fixed to the vertebral bone above and below but with a radiolucent discontinuity within the fusion mass; 2 = spondylodesis with solid bone bridging the fusion area.	Radiographic plain film and 3D CT: Fused/not fused (one reviewer)		Fusion defined as encapsulation of the HA block by bone and bone growth from superior to inferior vertebral body for the iliac crest bone graft specimens.
Histology	Decalcified (H&E stain)	Non-decalcified	Non-decalcified (Golder trichrome, H&E, or toluidine blue stains)	Undecacified (Villaneuva bone stain)	Non-decalcified (H&E, toluidine blue-O, and Villaneuva stains)	Decalcified & non-decalcified (modified tetrachrome stain)
Histologic Scoring	Histological scoring: 0 no evidence of new bone formation 1 fibrosis, osteoblast proliferation, minor new ossification; 2 mod, new, fibroendochondral ossification, partial fibrocartilaginous bridge; 3 fusion, fibrocartilaginous bone union to identifiable bone bridge	None	Fused/nonfused assessment	Fused/not fused	Bone apposition to the implant was assessed: 0, no tissue formation surrounding implant; 2, mild bone apposition and cartilage formation surrounding implants; 3, moderate bone apposition on 25%-50% of the implant surface area; 4, extensive bone apposition on more than 50% of the implant surface area (and further subgrouping into A, no evidence of bone incorporation into the pores; B, evidence of bone ingrowth into the pores)	Qualitative assessment only
Biomechanical Test Method	Manual palpation (single reviewer): 0 typical joint flexion–extension mobility; 1 reduced joint flexion–extension mobility; 2 rigidity, little to no joint flexion–extension mobility	None	None	None	Manual palpation (Absence of motion = fusion, any motion = nonfusion); Nondestructive testing using pure bending moments that were applied to the motion segments to induce flexion, extension, left and right lateral bending, and left and right axial rotation;	Nondestructive testing using pure bending moments that were applied to the motion segments to induce flexion, extension, left and right lateral bending, and left and right axial rotation;
Other Assessments	None	SEM Backscatter electron imaging; images were analyzed for composition of HA matrix, bone ingrowth, and soft tissue/vascular spaces	Histomorphometry (mean bone ingrowth, mean bone volume, mean MAR, mean MS/BS, Mean MFR); PLDLLA degradation assessment (qualitative); *in vitro* degradation analysis; Chemical analysis to assess crystallinity changes and polydispersity index.	None	None	Disc space height

TABLE X2.7 *Continued*

Category	Publication Reference					
Comments	Limitations: (*1*) Caution needed when extrapolating data to 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: (*1*) Caution needed when extrapolating data to 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: (*1*) Caution needed when extrapolating data to 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: (*1*) Caution needed when extrapolating data to 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: (*1*) Caution needed when extrapolating data to 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: (*1*) Caution needed when extrapolating data to 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.

TABLE X2.8 Published Examples for the Pig Posterolateral Spine Fusion Model

Category	Publication Reference	
Citation	Xue (251)	Christensen (250)
Breed	Danish landrace pigs	Göttingen mini-pigs
Chromosomal Sex	Female	Female
Age	12 weeks	24 months
Weight	~ 50 kg	~ 30 kg
Group Size (n)	N=11 (autograft control); N=11 (biphasic calcium phosphate)	N=18 (9/group)
Interbody Location	L2-L3, L5-L6	L3-L4
Control	Autogenous iliac crest autograft	Autogenous iliac crest autograft
Bone Graft Volume	4 g autogenous iliac crest autograft unilaterally; 8 g autogenous iliac crest unilaterally	Not specified
Bone Graft Material	Autograft; biphasic calcium phosphate	Autograft
Duration of Study	3 mo	3 mo
Radiographs	12 weeks	None
Radiographic Scoring	2 independent blinded physicians	None
Histology	Calcified, Golener's trichrome, flourochrome labeling	Calcified, basic fuchsin
Histologic Scoring	Blinded quantitative assessment of bone volume using the point-counting technique (To obtain random samples, a standard box with randomly distributed points was superimposed onto the sections under a light microscope. The box was moved up to down, down to up, and from right to left in turn for each column. Tissue volume identification was based on the number of points located at each tissue structure divided by the total number of points, which had been counted. Bone volume was calculated as percentage of the bone marrow, cartilage, and fibrous tissue in each fusion mass. A continuous trabecular bone bridging one adjacent transverse process to the next was identified as histologic fusion. The fusion rates and the volumes of fusion mass were compared.	Blinded quantitative evaluation of bone ongrowth was performed using the linear intercept technique, and a special software program (CAST-Grid, Olympus Denmark A/S, Glostrup, Denmark). Bone ongrowth was defined as bone in direct contact with the screw surface as a percentage of the total screw surface. Fibrous tissue and bone marrow with screw contact were also measured as percentage values. Bone ingrowth was defined as bone volume as a percentage of total volume. A line was drawn between each peak of the thread. Bone volume was counted as a percentage inside the thread enveloped by the line. Both bone ingrowth and ongrowth examinations were done in the body part according to the location of the spinal canal. The test systems for evaluation of bone ongrowth and ingrowth were calibrated to have approximately 200 intercepts or points counted for each parameter per specimen.

TABLE X2.8 *Continued*

Category		Publication Reference
Biomechanical Test Method	None	After sacrificing the animals, a torsion test was performed on the right-side screws and a pull-out test on the left-side screws. The specimens were thawed at room temperature, wrapped in latex to prevent cement from penetrating the bone, embedded in polymethylmethacrylate, and fixed into a metal holder. With the tissue block tilted to orient the screw axis vertically, the exposed end of the transpedicular screw was attached with two special adapters fixed to the upper load cell. The torque angle, pull-out force, load cell displacement and moments were recorded directly with a Teststar II acquisition system (790–10 Testware-SX Application, MTS Corp., Minneapolis, USA). The load-displacement data were analysed using NIH Image 1.51 producers and Excel 4.0 software producers. For the torsion tests, the screws were rotated 30° counter-clockwise at the speed of 0.5°/s. From the torsion tests, the maximum torque (Nmm) and angle-related stiffness (Nm/°) were calculated. For the pull-out tests, the screws were pulled out 10 mm at a rate of 0.2 mm/s. From the pull-out testing, the stiffness (N/mm), strength (N), and energy (Nmm) to failure of each screw were calculated. Stiffness was determined by calculating the slope of the early, linear portion of the load-displacement curve. To calculate the slope, a least-squares regression was performed on the raw data. Pull-out strength was defined as the maximum load to failure, and energy to failure was obtained by integrating the area under the load-displacement curve to the maximum load.
Other Assessments	Bone graft residual ratio/fusion mass volume (cm^3)/ autograft weight (g). The bone graft residual ratios were compared between the two groups and between the different amounts of bone graft.	Histomorphometry
Comments	Limitations: (*1*) Caution needed when extrapolating data to 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.	Pedicle screw fixation used; Limitations: (*1*) Caution needed when extrapolating data to 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.

TABLE X2.9 Published Examples for the Pig Interbody Spine Fusion Model

Category	Publication Reference			
Citation	Zou (271)	Li (272)	Zhang (270)	Ylinen (269)
Breed	Danish landrace pigs	Danish landrace pigs	Pig	Pig
Chromosomal Sex	Female	Female	Male	Not specified
Age	12 weeks	3 mo	Not specified (Adult)	Not specified (growing)
Weight	Not specified	~ 50 kg	50-60 kg	13.5-20.5 kg
Group Size (n)	N=11 (PT-ring+PSF); N=11 (PT-ring+ASF); N+11 (CF-cage+ASF)	N=12 (3/group)	Group 1 (rh-BMP-2 on a HA/TCP-collagen sponge, n = 6): final concentration of 0.43 mg/mL. The dosing of rhBMP-2 was 0.6 mg for each level. The HA/TCP-collagen sponge (15:85 HA/TCP ratio) was trimmed into 1.0 × 2.0 × 0.35 cm^3 strips, which is approximately 70% of the total volume of carrier that could be implanted with a given concentration of the rhBMP-2. Group 2 (iliac crest autograft, n = 4): right iliac crest bone was obtained through a separate fascial incision. A total of 6.0 cm^3 of corticocancellous bone was harvested, cleaned of soft tissues, and morselized. Group 3 (empty, n = 4): no graft material. Group 4 (HA/TCP-collagen sponge only, n = 4): 1.0 × 2.0 × 0.35 cm^3 HA/TCP-collagen sponge strips without rhBMP-2	N=21 implanted; N=4 control
Interbody Location	L2-L3, L4-L5, L6-L7	L3-L4, L4-L5	T5-T10	L4/L5 or L3/L4
Control	None	Autograft	Iliac crest autograft	Shams in 4 pigs in non-adjacent disc spaces
Bone Graft Volume	0.69g (PT-ring+PSF); 0.68g (PT-ring+ASF); 1.03 (CF-cage+ASF)	0.89 g (autograft); 0.80 mg (equine bone extract)	Group 1 (rh-BMP-2 on a HA/TCP-collagen sponge, n = 6): final concentration of 0.43 mg/mL. The dosing of rhBMP-2 was 0.6 mg for each level. The HA/TCP-collagen sponge (15:85 HA/TCP ratio) was trimmed into 1.0 × 2.0 × 0.35 cm^3 strips, which is approximately 70% of the total volume of carrier that could be implanted with a given concentration of the rhBMP-2. Group 2 (iliac crest autograft, n = 4): right iliac crest bone was obtained through a separate fascial incision. A total of 6.0 cm^3 of corticocancellous bone was harvested, cleaned of soft tissues, and morselized. Group 3 (empty, n = 4): no graft material. Group 4 (HA/TCP-collagen sponge only, n = 4): 1.0 × 2.0 × 0.35 cm^3 HA/TCP-collagen sponge strips without rhBMP-2	None, simply a reinforced HA block left empty

TABLE X2.9 *Continued*

Category	Publication Reference			
Bone Graft Material	Autograft	Autograft; COLLOS E (equine bone rextract)	rhBMP-2/ACS; Autograft; ACS	None
Duration of Study	6 mo	3 mo	4 mo	3, 6, 12, or 16 weeks
Radiographs	24 weeks	3 mo (plain and CT)	None	0 week; 3, 6, 12, or 16 weeks; microradiographs were also taken at sacrifice and evaluated to see fracture of the HA implant and incorporation of the bone inside the implant
Radiographic Scoring	None	None	None	Graded on scale of 1-4 (1 = 0% fusion to 4 = 76-100% fusion);
Histology	Calcified, basic fuchsin	Calcified, basic fuchsin	Undecalcified; Sanderson's Rapid Bone Stain	Masson modification of Goldner stain
Histologic Scoring	None	None		areas of HA, ingrown connective tissue, and ingrown new bone were measured
Biomechanical Test Method	None	None	None	None
Other Assessments	Histomorphometry	Histomorphometry; Blinded quantitative evaluation was performed using the points count technique; new bone volume was calculated as a percentage of the total volume inside the interbody fusion device	Histomorphometry: for each disc level, the original area of discectomy and remaining disc area were analyzed under light microscopy at 4× and 20× and defined by: (*1*) the remnants of the endplate; (*2*) junction of new bone formation/bone remodeling activity; and (*3*) residual necrotic bone graft or HA/TCP material. Total bone volume and fibrous tissue volume within both the original discectomy and remaining disc areas were measured for each disc level, respectively. In the remaining disc area, any ossification of the posterior anulus was reflected by both these indexes.	oxytetracycline (OTC) uptake was evaluated inside the implant as well as in the bone next to the implant. The uptake was also studied in the non-operated discs and in the facet joints
Comments	Pedicle Screw fixation and metallic interbody spacer used; Limitations: (*1*) Caution needed when extrapolating data to 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.	Pedicle Screw fixation and metallic interbody spacer used; Limitations: (*1*) Caution needed when extrapolating data to 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.	Pedicle Screw fixation used; Limitations: (*1*) Caution needed when extrapolating data to 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: (*1*) Caution needed when extrapolating data to 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.

TABLE X2.10 Published Examples for the Non-Human Primate Posterolateral Spine Fusion Model

Category	Publication Reference			
Citation	Boden (274)	Louis-Ugbo (285)	Barnes (286)	Boden (284)
Breed	Rhesus macaque monkey	Rhesus macaque monkey	Rhesus macaque monkey	Rhesus macaque monkey
Chromosomal Sex	Male	Not reported	Not reported	Not reported
Age	6-15 years	6-15 years	Not reported	6-15 years
Weight	13-18 kg	6-18 kg	Not reported	10-16 kg
Group Size (n)	N=8	N=8	N=9	N=16
Interbody Location	L4-L5	L4-L5	L4-L5	L4-L5
Control	DBM	None	None	None
Bone Graft Volume	3 g DBM (~10 cc) per animal; 3000 µg bone protein; 6000 µg bone protein, 10,000 µg bone protein	4 g autograft + Grafton Flex/side (N=4); 4 g autograft + Grafton Matrix (N=4) unilateral and 2 g autograft + Grafton Flex (N=4) unilateral	5.0 cc CRM; 5.0 cc CRM + 2.0 cc ACS	Ne-Osteo
Bone Graft Material	DBM; bone protein	Autograft; Grafton Flex; Grafton Matrix	rhBMP-2 + CRM; rhBMP-2 + CRM + ACS	
Duration of Study	12 weeks	24 weeks	24 weeks	24 weeks
Radiographs	12 weeks	0, 4, 8, 12, 16, 20, 24 wk (plain film); 24 wk (CT)	4-6 week intervals (plain film); 24 wk CT	24 week (plain film; CT)
Radiographic Scoring	None (blinded readings for fusion based on trabecular pattern)	Radiographs and CT scans were evaluated in a blinded fashion by two readers and graded semiquantitatively for the crosssectional area of the fusion mass (points were assigned: 3 = good, 2 = fair, 1 = poor). Also, for the extent of bone bridging between and incorporating into the transverse processes on each side (0: less than 25% of the distance between adjacent transverse processes, 1: 25–49%, 2: 50–74%, 3: 75–99%, 4: 100%). Points were then added for each site in each animal.	None	None (blinded readings for fusion based on trabecular pattern)
Histology	Non-decalcified histology (H&E stain or Goldner Trichrome)	Non-decalcified histology (Goldner Trichrome)	Methylene blue and basic fuchsin	Goldner Trichrome
Histologic Scoring	None	None	Histologic fusion was considered to be present if there was continuous new bridging bone across the carrier connecting the 2 transverse processes	None
Biomechanical Test Method	Manual Palpation	Manual Palpation	Manual Palpation	Manual Palpation
Other Assessments	None	None	None	None

TABLE X2.10 *Continued*

Category	Publication Reference			
Comments	Limitations: (*1*) Caution needed when extrapolating data to 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: (*1*) Caution needed when extrapolating data to 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. (*4*) Side-by-side design in the Matrix group used to assess the impact of the amount of autogenous bone graft implanted (*5*) Limited number of animals	Limitations: (*1*) Caution needed when extrapolating data to 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: (*1*) Caution needed when extrapolating data to 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. (*4*) Side-by-side design (*5*) Limited number of animals

TABLE X2.11 Published Examples for the Non-Human Primate Interbody Spine Fusion Model

Category	Publication Reference			
Citation	Luk (288)	Hecht (291)	Cook (293)	Steffen (292)
Breed	Rhesus macaque monkey	Rhesus macaque monkey	Pig-tailed macaque	Baboon
Chromosomal Sex	Male	Female	Not reported	Male
Age	4-6 years	Adult	10.7 ± 3 yr	Not reported
Weight	5.6-7.3 kg	4-6 kg	11.7 ± 3.5 kg	~25 kg
Group Size (n)	N=14	N=6	N=31	N=9
Interbody Location	L3-L4	L7-S1	L5-L6	L2-L3, L3-L4
Control	None	Autograft + cortical allograft cylinder	Autograft	Empty sham
Bone Graft Volume	Not reported	0.4 mg BMP (1.4 cc of Helistat)	Autograft; Autograft + bone growth stimulator	Not reported
Bone Graft Material	Autograft	Autograft; rhBMP-2 (0.270mL of 1.5 mg/mL solution) applied to 1.4 cc of Helistat.	Autograft	None
Duration of Study	2, 4, 6, 12 mo	6 mo	12 weeks; 26 weeks	6 mo
Radiographs	0, 2, 4, 6, 12 mo	0, 8, 14, and 18 wk (plain film); 2, 12, 24 mo (CT); 6 mo (microradiographs)	0, 12, 26 weeks (plain films, CT)	6 wk, 3 mo, 6 mo (plain film); 3 & 6 mo (CT)
Radiographic Scoring	None	None	0 No healing 0% (not healed) 1 Minimal consolidation of bone graft 1–25% healed 2 Consolidation of bone graft 26–50% healed 3 Bridging callus 51–75% healed 4 Bridging callus with trabeculations 76%–100% healed 5 Evidence of bony remodeling of callus NA	None
Histology	None	Undelcalcified; decalcified; Hematoxylin and eosin (H&E), Mallory-Heidenhain, toluidine blue O, and safranin O/fast green stains	basic fuchsin and toluidine blue stain	toluidine blue stain
Histologic Scoring	None	None	0: No healing 0% (not healed) 1: Minimal consolidation of bone graft 1–25% healed, 2: Consolidation of bone graft 26–50% healed, 3: Bridging callus 51–75% healed, 4: Bridging callus with trabeculations 76%–100% healed, 5: Evidence of bony remodeling of callus NA	Grading: I - fibrous tissue, II - fibro-cartilage, III - uncalcified bone matrix, IV -woven or parallel-fibered bone, V -remodeled cancellous bone)
Biomechanical Test Method	Flexion/extension static loading	Manual Palpation	None	None

TABLE X2.11 *Continued*

Category	Publication Reference			
Other Assessments	anterior, middle, and posterior disc heights were measured on the lateral radiograph using a computer digitizer; biochemical analysis of the intervertebral disc	None	None	None
Comments	Limitations: (*1*) Caution needed when extrapolating data to 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: (*1*) Caution needed when extrapolating data to 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.	Metallic interbody fusion device used; Limitations: (*1*) Caution needed when extrapolating data to 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: (*1*) Caution needed when extrapolating data to 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.

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