Designation: F3207 – 17

Standard Guide for *in vivo* Evaluation of Rabbit Lumbar Intertransverse Process Spinal Fusion Model[1]

This standard is issued under the fixed designation F3207; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 Historically, the single-level rabbit posterolateral, or intertransverse, lumbar spine fusion model was developed and reported on by Dr. Scott Boden, et. al. (Emory Spine Center for Orthopedics) and the model has been proposed as a non-clinical model which may be used to replicate clinically-relevant fusion rates for iliac crest autograft in the posterolateral spine (**1**, **2**).[2] This model is used routinely in submissions to regulatory bodies for the purpose of evaluating the potential efficacy of bone void filler materials as compared to other materials or iliac crest autograft to effect spinal posterolateral fusion. The use of this standard's recommendations as part of a regulatory submission does not provide any guarantee of regulatory clearance and should be considered as a part of the data provided for regulatory submission.

1.2 This guide covers general guidelines to evaluate the effectiveness of products intended to cause and/or promote bone formation in the lumbar intertransverse process spinal fusion model *in vivo*. This guide is applicable to products that may be composed of one or more of the following components: natural biomaterials (such as demineralized bone), and synthetic biomaterials (such as calcium sulfate, glycerol, and reverse phase polymeric compounds) that act as additives, fillers, and/or excipients (radioprotective agents, preservatives, and/or handling agents). It should not be assumed that products evaluated favorably using this guidance will form bone when used in a clinical setting. The primary purpose of this guide is to facilitate the equitable comparison of bone void fillers and/or autograft extender products *in vivo*. The purpose of this guide is not to exclude other established methods.

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

1.4 *This standard does not purport to address all of the safety concerns, if any, associated with the use of bone void fillers. It is the responsibility of the user of this standard to establish appropriate safety and health practices involved in the development of said products in accordance with applicable regulatory guidance documents and in implementing this guide to evaluate the bone-forming/promoting capabilities of the product.*

1.5 *This standard does not purport to address the requirements under 21 CFR Part 58 concerning Good Laboratory Practices or international standard counterpart OECD Principles of Good Laboratory Practice (GLP). It is the responsibility of the sponsor of the study to understand the requirements for conduct of animal studies whereby the data may be used to support premarket applications, including requirements for personnel, protocol content, record retention and animal husbandry.*

1.6 *This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the Development of International Standards, Guides and Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.*

2. Referenced Documents

2.1 *ASTM Standards:*[3]

E122 Practice for Calculating Sample Size to Estimate, With Specified Precision, the Average for a Characteristic of a Lot or Process

E1402 Guide for Sampling Design

E1488 Guide for Statistical Procedures to Use in Developing and Applying Test Methods

F2529 Guide for***in vivo*** Evaluation of Osteoinductive Potential for Materials Containing Demineralized Bone (DBM)

F2884 Guide for Pre-clinical ***in vivo*** Evaluation of Spinal Fusion

2.2 *Federal Documents:*[4]

21 CFR 58 Good Laboratory Practice for Nonclinical Laboratory Studies

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

[3] 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.

[4] Available from U.S. Food and Drug Administration (FDA), 10903 New Hampshire Ave., Silver Spring, MD 20993, http://www.fda.gov.

2.3 *AAMI/ISO Documents:*[5]

ISO 10993-6 Third edition 2016-12-01 Biological evaluation of medical devices — Part 6: Tests for local effects after implantation

3. Terminology

3.1 *Definitions:*

3.1.1 *biomechanical fusion, n*—the increased strength and/or stiffness and reduced ROM of a spinal unit as compared to that measured before surgical intervention.

3.1.2 *biomechanical properties, n*—as used in this document, evaluation of the operative functional spinal unit multidirectional range of motion (ROM: Lateral bending, Flexion – Extension and Axial Rotation) properties under non-destructive conditions, tensile stiffness and ultimate load.

3.1.3 *fusion, n*—a multifactorial outcome which can be characterized in terms of the radiographic, biomechanical and histological results of the intended spinal arthrodesis procedure.

3.1.4 *histological evidence of fusion, n*—based on light microscopy of newly formed and remodeled bone spanning the intertransverse region, with contiguous osseous connectivity observed between the adjacent transverse processes. Assessment rationale must be justified.

3.1.5 *manual palpation, n*—a method for evaluating spinal fusion status by estimating the stiffness of the operative motion segment and adjacent superior motion segment by the application of multidirectional loads in lateral bending and flexion-extension using the hands.

3.1.6 *micro-computed tomographic (micro-CT) fusion, n*—tomographic fusion is based on interpretation of the micro-CT images, with fusion success based on the three-dimensional appearance of contiguous bone from transverse process to transverse process (i.e. bridging bone).

3.1.7 *non-union, n*—a multifactorial outcome which can be characterized in terms of the radiographic, biomechanical and histological results indicating a lack of trabecular or cortical bone spanning the intertransverse region, without contiguous osseous connectivity observed between the adjacent transverse processes.

3.1.8 *radiographic fusion, n*—status of radiographic fusion is based on interpretation of the posteroanterior (P/A) plain film x-ray images, with fusion success based on the appearance of contiguous bone from transverse process to transverse process (i.e. bridging bone).

4. Significance and Use

4.1 This guide covers animal implantation methods and analysis of bone void fillers to determine whether a material or substance leads to lumbar intertransverse process spinal fusion, as defined by its ability to cause bone to form *in vivo*.

[5] Available from Association for the Advancement of Medical Instrumentation (AAMI), 4301 N. Fairfax Dr., Suite 301, Arlington, VA 22203-1633, http://www.aami.org.

5. Animal Models

5.1 *General Note*—Appropriate positive or comparative controls may be used. For example, comparative controls could be similar devices, and positive controls could be autograft from the animal.

5.2 *Skeletally mature New Zealand white rabbits*—(typically > 7 months and 3.5-4.5kg). Proximal tibial and distal femoral physes should be closed and verified via plain radiographs. Radiographic and histologic closure of the distal femoral growth plates occur at an average age of 21 and 22 weeks, respectively. The proximal tibial physes close radiographically and histologically at an average age of 26 and 28 weeks, respectively. A lateral radiograph is a more reliable method for assessing physeal closure in the rabbit, and radiographic confirmation of tibial physeal closure should be obtained prior to using rabbits that are younger than approximately 7 months of age. Radiographic confirmation of physeal closure is probably not necessary in rabbits 8 months or older but should be provided for the sake of completeness. Some minor variation in age of tibial growth plate closure may be expected with different strains of New Zealand White rabbits. Weight is not a reliable indicator of skeletal maturity in the New Zealand White rabbit. All rabbits used should be of the same sex. **(3, 4)**

5.3 *Implant Mass/Volume*—In general, implant mass (~1.6-2.2 grams; useful only for autograft)/volume (~2.5-3.0 cc) per side is used. It is recommended that the experimental group contain the same total implant volume as any comparative groups so the results are comparable and the potential effects of the implant on intertransverse process spinal fusion can be determined.

5.4 *Sample Size:*

5.4.1 Sample sizes should be justified in the study protocol and, if possible, should provide statistical power appropriate to the endpoint using appropriate statistical methods to justify as required. Interim time points may be used as appropriate and justifications should be provided. Should statistical numbers not be practical or possible, empirical testing in the literature has shown an n=6-8 to be a target sample size minimum.

5.4.2 Sample size should be determined with reference to the primary outcome of the study, which is typically the fusion rate at 8 or 12 weeks. Additionally, it may be necessary to consider the sampling requirements of other analyses in the study; in particular, quantitative endpoints such as morphometry.

NOTE 1—The sample size recommendations refer to the number of samples expected to be available for analysis. Attrition, or loss of animals due to surgical complications, is common in the rabbit spinal fusion model (especially with autograft harvesting). It may be necessary to plan for additional animals to replace those lost to attrition. Make sure you report all animals treated, any unexpected or early deaths, etc.

5.5 *End Points*—Each implant group should have an immediate post-operative assessment and end points should be justified by the resorption profile of the materials; there should be at least 2 time points less than the maximum assessment time (an early and mid-phase) in order to assess any irregularities (unexpected or excessive inflammation, etc.) at the

implant and peri-implant site (recommended time periods representative in the literature are 4, 8, and 12 weeks) or longer periods may be warranted and should be justified.

6. Recommended Surgical Protocol Methodologies

6.1 *Rabbit Lumbar Intertransverse Process Spinal Fusion Recommended Surgical Technique:*

6.1.1 Aseptic technique should be employed during the surgical implantation procedures.

6.1.2 Animals should be singly housed in standard cages and fed with rabbit food and water.

NOTE 2—Handling of the animals during the first 14 days post-op should be avoided unless medically required.

6.1.3 Pre-operative analgesics: 0.05 mg/ kg buprenorphine administered subcutaneously and the application of a fentanyl patch (25 µg/hr) to the inner ear pinna, or other analgesic approved by the IACUC. A 25 µg/hr fentanyl patch is an effective analgesic with duration of up to 72 hours, but may require up to 12 hours after application until blood levels are sufficient to provide pain relief. Patches may be placed the evening prior to surgery or animals dosed with an analgesic such as butorphanol prior to surgery and several hours after surgery to ensure analgesisc coverage while fentanyl blood levels rise.

6.1.4 Anesthetics: Induction and maintenance: 34 mg/ kg ketamine and 5 mg/ kg xylazine administered intramuscularly. Isoflurane should be administered via laryngeal mask within a range of 2%-3%, but increasing and decreasing the percentage administered should be based on the individual animal response. Ophthalmic ointment should be applied to the eyes following pre-anesthesia and prior to surgery.

6.1.5 Identify each animal with a unique identifier (ear tag, tattoo, etc.). Record the individual animal identification numbers along with the body weights.

6.1.6 Sedate the animal with an IACUC approved medication and maintain general anesthesia with Isoflurane or other anesthetic approved by the IACUC. The depth of anesthesia should be sufficient to prevent muscular movement. This can be checked by pinching the toe (between the digits) of the animal's hind limbs. If there is a reflex reaction, the animal is not sufficiently anesthetized to continue with the implantation. A technician shall monitor the animal's vitals/parameters while under anesthesia and record every 15 minutes.

6.1.7 Place the anesthetized animal in a sternal or ventral recimbant position on a clean flat surface in a procedure room and shave the dorsum of the animal from the mid thoracic region well below the iliac crests with clippers. Scrub the clipped area with surgical scrub (chlorhexidine scrub or povidone scrub). Start from the center and work, in a circular fashion, to the edge of the surgical area. Wipe off the surgical scrub with 70 % isopropyl alcohol (repeat entire scrub procedure at least 3 times). The surgeon will complete final preparation for aseptic surgery.

6.1.8 Transfer the anesthetized animal to the surgical suite.

6.1.9 Lumbar Posterolateral Intertransverse spinal fusion is detailed as follows:

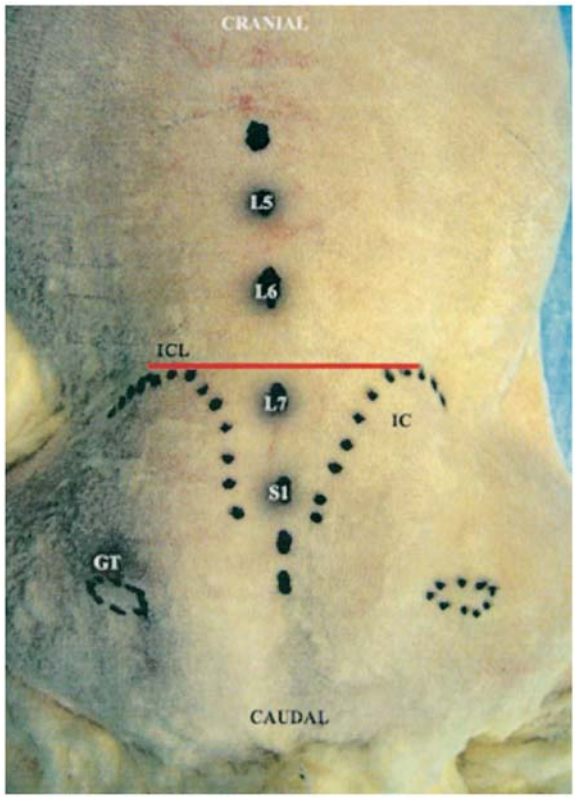
6.1.9.1 Final sterile prep of the surgical site is completed in the operating room with 2% chlorhexidine or povidone solution prior to first incision. Start from the center and work to the edge of the surgical area. Wipe off the solution with a clean, sterile gauze pad. The spinal level to be fused, most commonly L4–L5 or L5–L6, is then identified by palpation. A line drawn from the most cranial aspect of one iliac crest to the other, the intercrestal line, will generally pass between the L6 and L7 spinous processes (Fig. 1). A second method to verify the correct operative level is based on the anatomy of the lumbosacral spinous processes. Specifically, there is often a much wider interspinous distance at L6–L7 than there is at L5–L6, L7–S1 or between the sacral processes (Fig. 1), although this is not always the case.

6.1.9.2 Using both techniques of localization, the L4–L5 or L5–L6 level can be correctly identified in the vast majority of animals. Errors can occur, however, because of the presence of osseous anomalies of the lumbosacral vertebrae. A preoperative dorsoventral radiograph is advisable. In the presence of an abnormality, which alters the typical number of lumbar motion segments, the animal should be excluded or the spinal level just cranial to the intercrestal line can be used.

6.1.9.3 It is acceptable to perform the surgery at either L4–L5 or L5–L6, vertebral levels, although the choice of level should be consistent within the study. Variability in the number of lumbar vertebrae is common in certain strains of NZW rabbits-some rabbits exhibiting 6 lumbar vertebrae while others have 7. In such cases, pre-operative radiography is advisable to positively identify the target operative site. Performing surgery at L5–L6 in such populations will result in the operative space adjacent to the lumbosacral space in some rabbits and at one space proximal in other cases. It is unknown if the biomechanical forces across the inter-lumbar joints are all equal or if there are differences between the joint adjacent to the lumbosacral joint and more proximal joints. Selecting L4–L5 as the operative site may minimize this potential problem, as L4–L5 is separated from the lumbosacral joint by at least one motion segment. Fusion masses at L4–L5 may be easier to harvest than a fusion mass at L5–L6 in cases where there are only 6 lumbar vertebrae. Finally, in many cases where there are only 6 lumbar vertebrae, the 6th transverse process may be quite narrow than the transverse process of L4 or L5. There may be no significance to this observation.

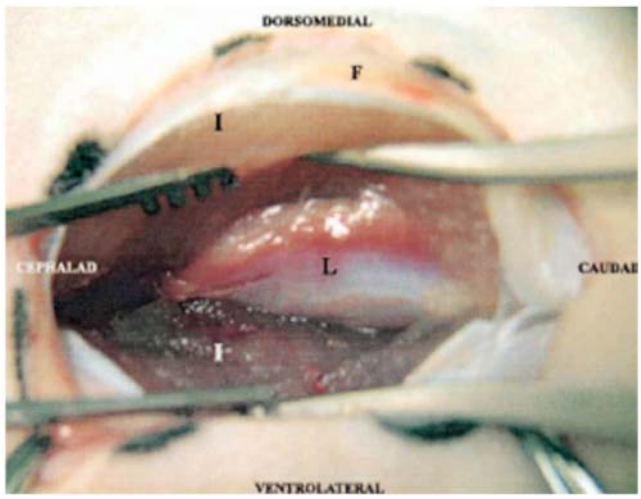
6.1.9.4 *Surgical Approach*—A representative description assuming an L5–L6 fusion site is described hereafter. A dorsal midline skin incision measuring approximately 6 cm in length is centered over the L5–L6 level. A full-thickness flap of skin and subcutaneous tissue is developed and retracted to one side. Approximately 2 cm lateral to the midline at the L5–L6 level, a 4-6 cm longitudinal incision is made through the lumbar fascia. Through this fascial incision, the iliocostalis muscle is divided exposing the underlying longissimus muscle (Fig. 2).

(1) To reach the transverse processes, blunt dissection is performed along the lateral border of the longissimus muscle. Exposure of the posterolateral fusion site is accomplished by elevating the iliocostalis muscle in a lateral direction off the transverse processes and intertransverse ligament. Dorsomedial retraction of the longissimus muscle is required to expose the medial aspect of the transverse processes and the pars interarticularis. Care should be taken to avoid inadvertent



NOTE 1—Dorsal view of the rabbit lumbosacral region. The iliac crests (IC) and the greater trochanters (GT). The intercrestal line (ICL), drawn from the most cranial aspect of one iliac crest to the other, will generally pass between the L6 and L7 spinous proceses. The interspinous distance at L6–L7 is substantially wider than at L5–L6 and L7–S1.

FIG. 1 Localization of the L5–L6 Fusion Site

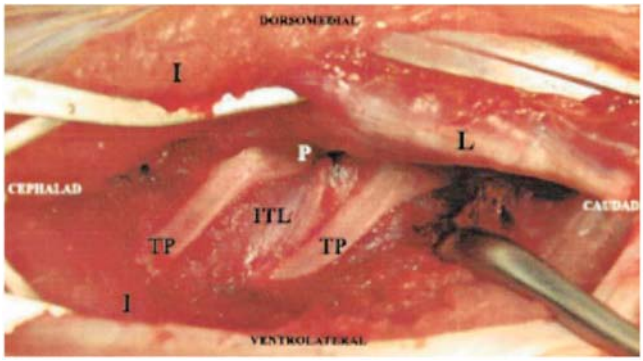


NOTE 1—After mobilizing the skin, the lumbar fascia (F) is vertically incised approximately 2 cm lateral to the spinous processes at the L5–L6 level. Through this fascial incision, the iliocostalis muscle (I) is divided exposing the underlying longissimus muscle (L).

FIG. 2 Surgical Approach: Superficial Dissection

exposure of adjacent facet joints or transverse processes at the proximal and distal levels. A small self-retaining retractor will maintain exposure of the two transverse processes and the intertransverse ligament (Fig. 3).

(2) To minimize bleeding during and after surgery, the dorsal branch of the segmental artery is cauterized as it passes with the posterior ramus through the operative field. As a means of limiting hemorrhage, it is also helpful to pack the wound with gauze upon completing the first surgical approach. After exposure and packing of the contralateral fusion site, retractors are replaced on the initial side to begin the decortication process. It is advisable to simultaneously palpate the left and right fusion sites to verify that the same level has been exposed on both sides of the spine.



NOTE 1—To reach the transverse processes, blunt dissection is performed along the lateral border of the longissi- mus muscle. Exposure of the posterolateral fusion site is accomplished by elevating the iliocostailis muscle (I) off the transverse processes (TP) and intertransverse ligament (ITL) and then retracted ventrolaterally. Dor- somedial retraction of the longissimus muscle (L) is required in order to expose the pars interarticularis (P).

FIG. 3 Surgical Approach: Deep Dissection Demonstration to Show the Anatomy

(3) Decortication of the transverse processes is performed with a motorized burr until punctate bleeding observed. Transverse process decortication should be performed as indicated by Fig. 4. The extent of decortication has been shown to be a determining factor in fusion rate, so care should be taken to ensure that decortication does not extend on to the vertebral body, as this may result in higher than expected fusion rates.

(4) The fifth lumbar root is vulnerable to injury as it exits the L5–L6 intervertebral foramen immediately dorsal to the plane of the intertransverse ligament and transverse processes. The lumbar plexus is also vulnerable to injury as its component nerves pass just ventral to the intertransverse ligament making it essential to preserve the integrity of this ligament during the exposure and decortication process.

6.1.9.5 *Harvest of Iliac Crest Bone*—Arthrodesis using autogenous bone from the ilium is often implemented as a control group in spinal fusion research using the NZW rabbit model. Working through the same dorsal skin incision, the cranial and lateral surface of the iliac crest is exposed in a subperiosteal plane (Fig. 5, top). This central part of the iliac wing contains the greatest amount of cancellous bone and can be localized by palpation of the medial iliac spine (Fig. 5, bottom). The recommended quantity of graft, 2.5 to 3.0 cc per side of the spine, generally requires harvesting a significant proportion of both ilia. During graft harvest, it is critical to be gentle when elevating the muscles off the inner cortex when taking the tricortical ilac crest graft. Dissection in this area can traumatize the neurovascular structures that pass through the sciatic notch leading to serious hemorrhage and/or sciatic nerve palsy. Some amount of palsy (~10%) is an expected consequence of harvesting the recommended 2.5 to 3.0 cc of graft.

6.1.9.6 Morselize the corticocancellous autograft bone with a rongeur into <5 mm irregular pieces. Make sure to remove all soft tissues from the morselized iliac crest bone.

6.1.9.7 Decortication and grafting material should be confined to the medial one half of the two transverse processes (i.e., half of the transverse process that is close to the vertebral body). As shown in Fig. 4, the graft material should be placed on top of the red zones and filled in between the two transverse processes. Place either the iliac bone autograft or the test article between the transverse processes in the paraspinal bed, paying particular attention to placing the graft material along the medial half to one-third of the transverse processes where decortication was done (Fig. 6).

6.1.9.8 Close the fascial incisions with 3-0 absorbable suture and the skin edges are approximated using absorbable 3-0 or 4-0 suture, with or without 35W staples.

6.2 *Recovery—Post-operative Care and Analgesics:*

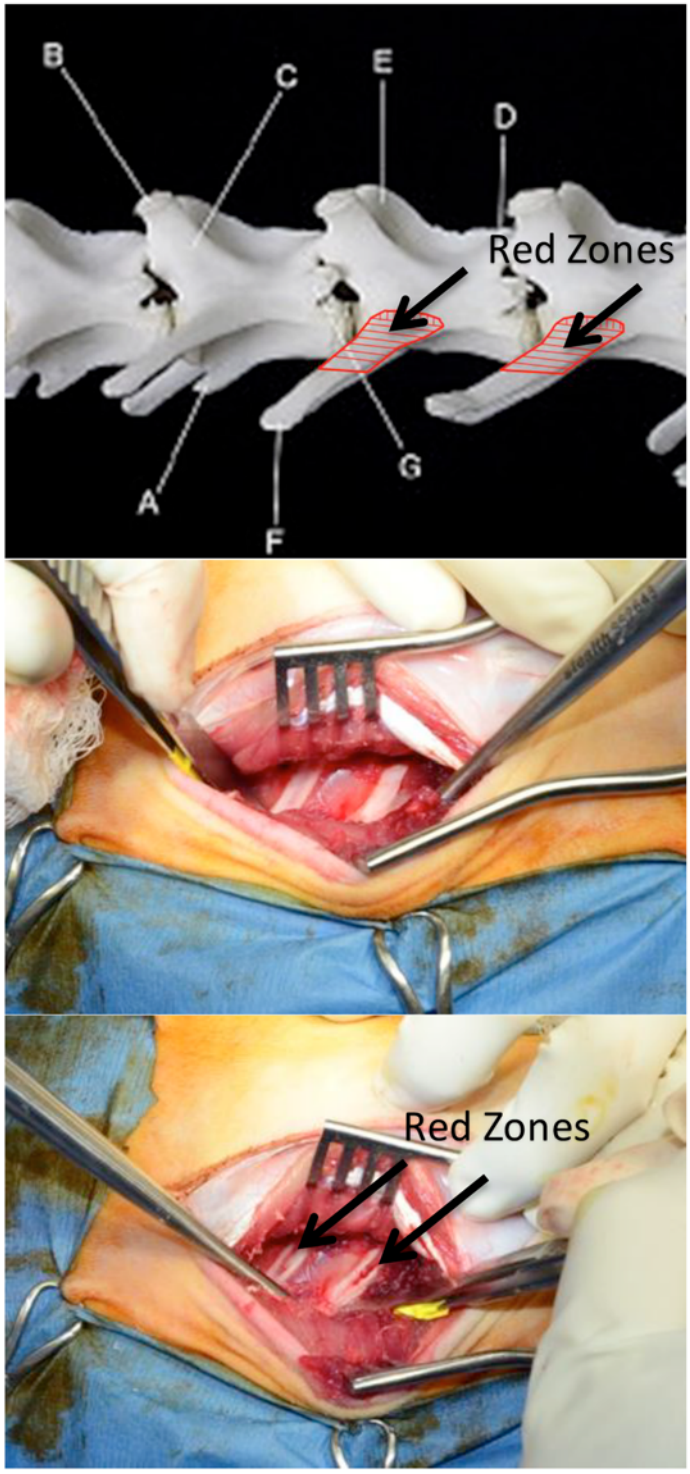
6.2.1 Warm blankets and heated mats should be used both intra-operatively and post operatively to keep the animal's body temperature within normal range. For analgesics, 0.05 mg/ kg buprenorphine should be administered subcutaneously approximately 6 hours after the first dose. The second dose of buprenorphine should give the animals an adequate plane of analgesia until the fentanyl patch reaches therapeutic levels. Fentanyl patches should be replaced approximately every 72 hours until the animal is no longer deemed painful. Pain levels can be monitored based on how well the animals are eating, posture and ease of movement within the cage. When these 3 observations are deemed normal, the animal can then be considered pain free. If not all of those items are normal, then consideration needs to be given for additional analgesics.

6.2.2 During the first hour after surgery, pulse and respiratory rate are monitored; supplemental fluids are administered intravenously or subcutaneously as needed. Animals should be monitored until ambulatory, handled carefully, and then returned to their cage.

6.3 *Post-operative Care:*

6.3.1 The general condition of the rabbit should be monitored twice each day for the first 3 days after surgery, followed by once a day for the remainder of the study. If used, skin staples are removed 2 weeks after the operation.

6.3.2 Post-operative anorexia in rabbits may be a serious complication and can result in death within 4-6 days. To that end, rabbits should be encouraged to eat after surgery. Rabbits may be supplemented with fresh fruits or vegetables (apples, carrots, timothy hay cubes) during the acute post-operative period if dietary intake of their normal ration is reduced. A particularly effective supplement is Critical Care by Oxbow Animal Health, which is a highly palatable, high fiber supplement for herbivores that is highly effective in stimulating



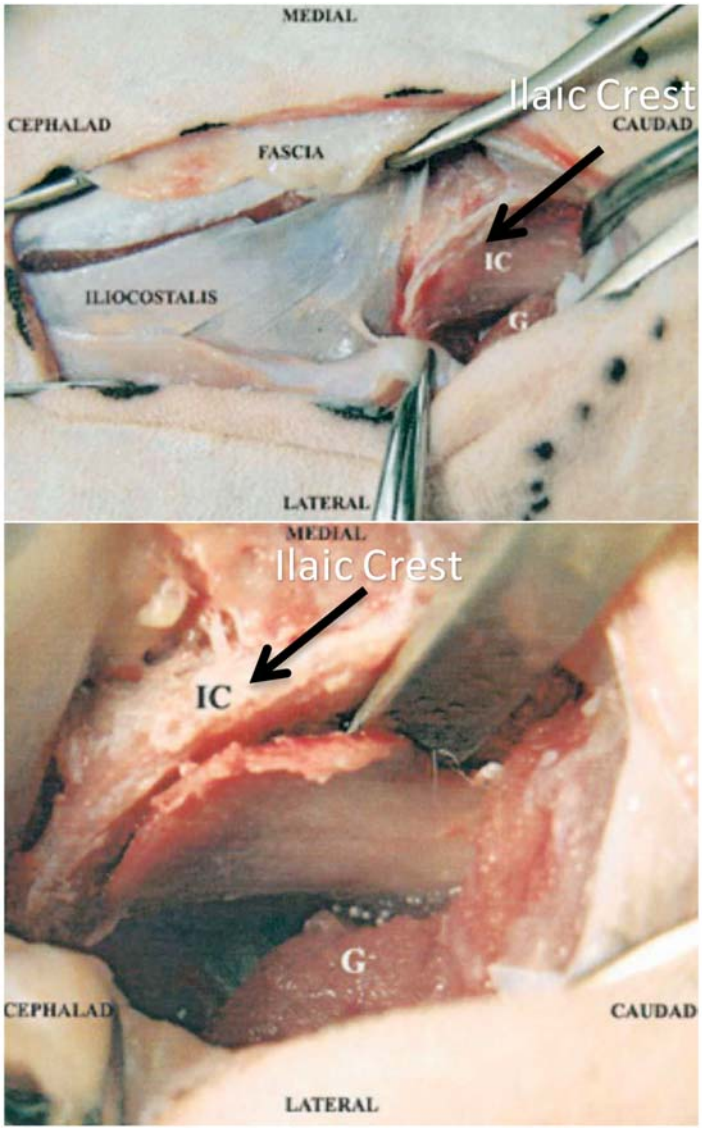
NOTE 1—(Top) Schematic representation of the decortication area. (Middle) Virgin transverse processes. (Bottom) Decorticated Transverse processes.

FIG. 4 Decortication of Transverse Processes

appetite in post-operative rabbits. Rabbits are weaned off supplements as they regain their appetite for their normal ration.

6.4 *Recommended Observations:*

6.4.1 *General Health*—Observations can occur through close, cage-side observations. If any abnormal clinical signs including signs of inflammation and/or infection, hind limb



NOTE 1—(Top) Working through the same skin incision, the cranial and lateral surface of the iliac crest (IC) is exposed by elevating the gluteal musculature (G) in a subperiosteal plane. (Bottom) The greatest amount of cancellous bone can be harvested from the central part of the iliac wing.

FIG. 5 Harvest of Iliac Crest Bone Graft

paresis, decreased food and water intake or decrease urine and fecal output are observed, inform a staff veterinarian and/or designated personnel.

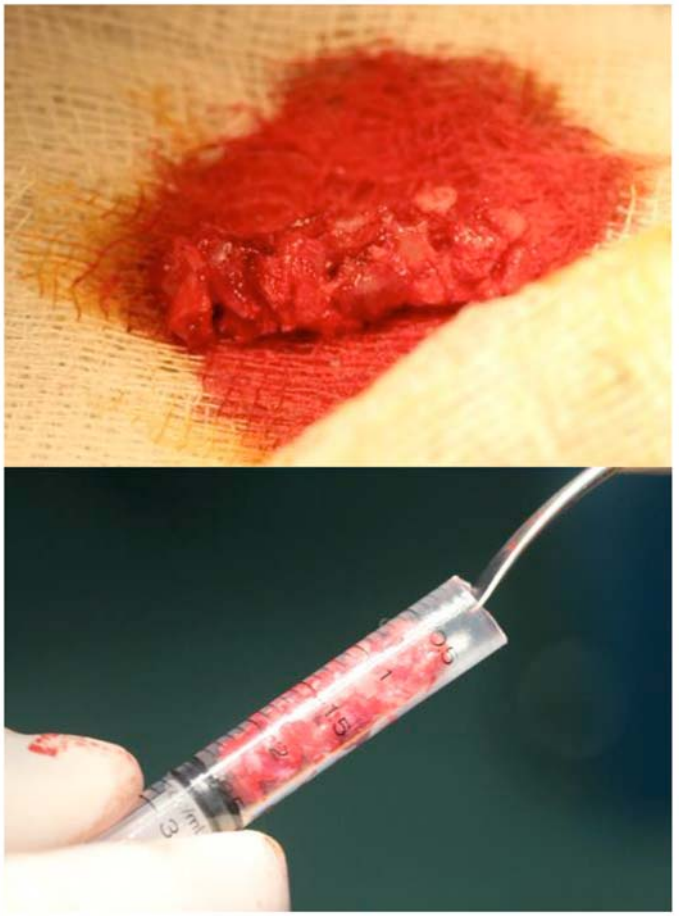
6.4.2 *Early Deaths*—If a rabbit should die during the study, perform a necropsy on the animal. If the animal died on or after day 14 of the study, harvest and fix the implant sites until a decision is made about the potential utility of processing tissues for histopathology. If the animal dies before day 14, perform a necropsy and contact the staff veterinarian and appropriate personnel. A terminal weight should always be recorded.

6.5 *Recommended Euthanasia*—At the end of the implantation period, record the terminal weight of each animal. Record any abnormalities, with respect to the animals' health, on the macroscopic observations section of the animal record. Euthanize the animals using an IACUC-approved method consistent with AVMA Guidelines on euthanasia.

7. Experimental Endpoints: Techniques to Analyze New Bone Formation

7.1 *Animal Necropsy:*

7.1.1 Test samples immediately when possible (i.e. not ever frozen). It is possible, but not recommended, to store samples frozen. If they are frozen then all samples in the study should be treated the same way. If samples have not been tested immediately after necropsy, storage conditions (e.g., frozen, etc.) should be recorded in the test report. With materials that do not have a long history of use, it is advisable that select systemic tissues and any gross lesions should also be excised and preserved using fixation methods appropriate to the experiment's requirements. These tissues may include but are not limited to the following: heart, kidneys, liver, lungs, spleen, pancreas, axillary lymph nodes, mesenteric lymph nodes, periaortic lymph nodes, and a sample of local paraspinal tissue sample overlying the fusion site. Following careful posterior



NOTE 1—(Top) Weight may be determined following removal of any excess blood / fluids with gauze. (Bottom) Volume may be determined by packing into an open bore syringe under light compression.

FIG. 6 Iliac Crest Autograft is Morselized to Approximately <5 mm in Size

dissection of the paraspinal tissues, the spinal column from the mid-thoracic region through the pelvis should be excised en bloc. The operative lumbar spine and operative site should be examined for gross evidence of infection or general inflammation. A full gross necropsy should also include examination of external surfaces/orifices of the body, the cranial, thoracic, abdominal cavities, and all viscera.

7.1.2 The following list suggests a possible order of procedures to be performed post-necropsy. The test report should list and justify the order of the procedures done during the experiment. Careful attention should be paid to this order so that experimental results are not compromised by previously performed procedures. It is recommended that the researchers note why the results of all sample testing is valid and not compromised by previous testing done on the samples.

7.1.2.1 Systemic and reticuloendothelial tissues harvest and lumbopelvic spine resection

7.1.2.2 Plain film radiography of the lumbar spine (see Note 3)

7.1.2.3 Micro CT (see Note 3)

7.1.2.4 Manual palpation

7.1.2.5 Biomechanical testing (see Note 4)

7.1.2.6 Histology and histomorphometry (see Note 4)

NOTE 3—It is recommended to complete all radiographic tests prior to biomechanical testing.

NOTE 4—It is recommended that specimens used for destructive biomechanical testing NOT be used for histological or histomorphometry testing as destructive biomechanical testing performed on the specimens may introduce artifacts in further tests.

8. Techniques for Assessing Fusion

8.1 *Radiographic Analysis:*

8.1.1 *Obtaining Radiographs of the Operated Site*—Obtain dorsoventral plane film radiographs of all operated segments immediately post-op and at the final study time point after animal sacrifice. It is recommended that the radiographic equipment set-up be consistent for each assessment. Radiographs should be clear with minimal artifacts and should enable visualization of the entire operated segment and at least one (1) caudal and cranial non-operated level. Animal designations and orientation using standard markers should be employed.

8.1.2 *Personnel*—The presence of radiographic fusion will be scored by three (3) independent observers. These personnel must be trained in the technique and be blinded to the treatment

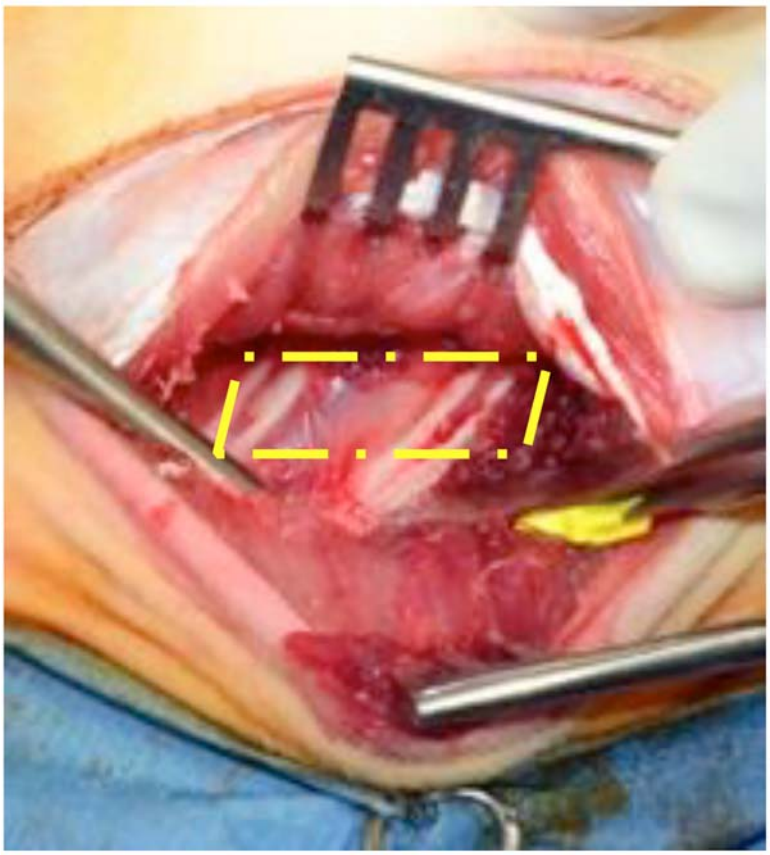


FIG. 7 Approximate Graft Placement Over the Paraspinal Bed and Adjacent Transverse Processes

group. Scoring is aided by knowledge of the post-op radiographic appearance of the operated site and bone graft material. When possible, the post-op film should be made available during scoring.

8.1.3 *Radiographic Scoring:*

8.1.3.1 Scoring of radiographs for fusion should be conducted at pre-defined analysis time points. Each side (left and right) of each operated level should be independently scored and reported for fusion. A side is scored as "fused (F)" if continuous radiopaque bridging bone is visible between adjacent transverse processes of the operated segment. A side is scored as "not fused (NF)" if radiolucent features are present that prevent continuous radiopaque bridging of bone between adjacent transverse processes of the operated segment.

8.1.3.2 Fusion results may be summarized by animal, where an individual animal is considered bilaterally fused if both sides are scored as fused (F/F), unilaterally fused if only one side is scored as fused (F/NF), or non-fused if neither side is scored as fused (NF/NF).

8.1.3.3 The report should include names of personnel who performed the assessment, their blinding to treatment group, and final result of fused or non-fused for the affected fusion level. The grade of all observers should be documented and reported.

NOTE 5—This assessment of radiographic fusion as defined by bridging bone does not preclude additional observation or grading of other fusion mass characteristics which may be appropriate depending on the specific scientific questions of interest. Additional characteristics visible by radiography may include fusion mass size and/or maturity (presence of more organized trabecular structures and a neocortex) and resorption of radiopaque bone graft materials. However, alternative methods (e.g. micro-CT or histomorphometry) may quantitatively evaluate these characteristics with sensitivity beyond plain film assessment.

NOTE 6—Assessing radiographic fusion of radiopaque bone graft materials can increase false-positive fusion rate reporting. If the observer is unable to distinguish bridging bone from residual radiopaque bone graft material at the operated site, the side must be scored "indeterminate – obscured view." Radiographic fusion rates for radiopaque bone grafts should be clearly disclosed in reports and the limitations of this analysis should be detailed. Additional techniques for fusion assessment must be conducted to substantiate fusion of radiopaque bone graft materials.

8.2 *Micro Computed Tomographic Analysis:*

8.2.1 Micro computed tomography may be performed to obtain high resolution images of the bone formation and implant resorption and should be performed prior to biomechanical testing. This imaging modality may provide greater resolution than plain radiographs, and provides multiple sections in multiple planes (axial, sagittal, and coronal), as well as three-dimensional reconstruction of the entire fusion mass. Required resolution of the scans will be dependent on the characteristics of the implants, in order to differentiate between bone and implant (<96 microns is a good guideline, suggested 50 microns at a minimum). Micro computed tomographic images may be scored from the sagittal and coronal planes using the same criteria for continuous radiodense bridging bone as used for plain radiographs. Each side (left and right) of each operated level should be independently scored and

reported for fusion. Fusion success may be calculated according the same analysis method used for plain radiographs.

8.2.2 Additionally, micro-CT images may be used to quantitate morphometric parameters including total fusion mass volume (cc), mineralized bone volume (cc), and residual implant material volume (cc). The transverse processes should be excluded from the volumetric analyses.

8.2.3 Note that implants which contain radiopaque materials may interfere with the fusion assessment and quantitative measurement of mineralized bone volume.

8.3 *Manual Palpation:*

8.3.1 *Primary Biomechanical Assessment Option:*

8.3.1.1 Manual Palpation is the most accepted method used for non-destructive mechanical assessment of posterolateral fusion in this model.

8.3.1.2 Other methods of biomechanical assessment (described in section 8.4) may be used in addition to manual palpation in order to gain further insight into assessment of the fusion site.

8.3.2 *Minimization of Bias*—Manual palpation can be a reliable technique to determine fusion. Determination of fusion by this method is subjective therefore methods to minimize bias must be employed. Examiners must be blinded to treatment group and assessment should be done in a random fashion.

8.3.3 *Personnel*—Stiffness of the fused motion segment will be assessed by manual palpation by three (3) independent observers. These personnel must be trained in the technique and be blinded to the treatment group. It is best practice to perform manual palpation on multiple specimens at one time in a blinded fashion.

8.3.4 *Timing of Stiffness Assessment*—Manual palpation is best performed soon after necropsy (< 60 min) or, if specimens have been frozen and thawed to room temperature, the specimens must be checked to confirm adequate thawing and the method of determination should be documented. Regardless, all samples should be treated in an equivalent fashion.

8.3.5 *Harvesting and Cleaning of Specimen*—Three motions segments, the level of interest and one above and below should be harvested together after euthanasia. The soft tissue around the spine should be removed unless need for histologic analysis to expose the dorsal and ventral regions between the transverse processes and the intervertebral disc. This aids in the ability to grasp the vertebral body and spinous processes to apply bending moments.

8.3.6 *Palpation:*

8.3.6.1 Manual bending force will always be applied to three levels of the spine during the palpation process. The levels immediately cranial and caudal to the surgical site (assuming an L4-L5 fusion site, e.g. L3-4, L5-6) will be palpated first to ensure sufficient bending force is applied to elicit motion. The operated level will then be palpated using similar force.

8.3.6.2 Assuming fusion was attempted at L4-L5, palpation will be accomplished by grasping the spine at L5 with the thumb and index finger of one hand and L4 with the thumb and index finger of the other hand. Mild pressure will be applied in lateral bending in one direction and then the other and the presence of motion in the segment determined by direct visualization of the ventral aspect of the spine (vertebral bodies and transverse processes). Mild bending pressure will then be applied in flexion-extension and lateral bending. If any part of L4 (transverse process, vertebral body) or across the intervertebral disc moves relative to L5 (or vice versa) during the application of bending force, the segment will be graded "M" for "motion". If no motion is observed during the application of bending force, the segment will be considered to be fused and graded as "F". The spinal unit should be graded as a whole and the grade of all observers should be reported.

8.3.7 *Definition of Fusion*—Fusion for manual palpation assessment is defined as no movement at the affected fusion level under physical manipulation or palpation.

8.3.8 *Reporting*—The report should include names of personnel that removed the spines after necropsy, who performed the assessment, their blinding to treatment group, the result of manual flexion-extension and lateral bending for each side, and final result of fused or non-fused for the affected fusion level.

8.4 *Biomechanical Testing:*

8.4.1 *Uniaxial Tensile Testing:*

8.4.1.1 Uniaxial tensile testing is a simple way to determine the mechanical yield strength and stiffness of the treated level of spine versus the adjacent untreated level of spine.

8.4.1.2 Samples for biomechanical testing should be stored frozen until the evening before testing. If specimens have been frozen and thawed to room temperature, the specimens must be checked to confirm adequate thawing and the method of determination should be documented. Regardless, all samples should be treated in an equivalent fashion.

8.4.1.3 Immediately before mechanical testing, all remaining muscle as well as the facet joints should be removed with a rongeur.

8.4.1.4 The intervertebral disc should then be divided with a scalpel so that only the fusion mass and the intertransverse membrane were left to connect the two fused vertebrae.

8.4.1.5 Uniaxial tensile testing should be performed at a displacement rate of 0.5 cm/min. Apply the load to the motion segment of interest (generally L4–L5 or L5–L6) via two 3.2 mm diameter steel rods drilled from ventral to dorsal through L5 and L6 vertebral bodies respectively.

(1) Load/displacement data should be continually generated and recorded digitally using a computer and graphically using an x-y recorder.

(2) Ultimate tensile load should be read directly as the peak load to failure.

(3) Stiffness should be calculated as the slope of the linear portion of the load/displacement curve.

8.4.1.6 The adjacent unfused L3–L4 or L4–L5 motion segment in each rabbit should be tested in an identical manner in order to obtain an internal control to minimize the effect of biologic variation among animals.

8.4.1.7 *Results*—The ultimate tensile load and stiffness of the fusion level (L4–L5 or L5–L6) and the adjacent control level (L3–L4 or L4–L5) should be reported. The ratio of the ultimate tensile load and stiffness of fusion to control level should also be reported.

8.4.1.8 *General Note*—Because of the potential for tissue damage, this type of testing should not be performed on samples intended for histological assessment.

8.4.2 *Quantitative Multidirectional Flexibility Testing:*

8.4.2.1 Quantitative multidirectional flexibility testing may be performed to assess the operative functional spinal unit range of motion properties. This serves to quantify and compare the motion characteristics of the posterolateral treatment procedures using quantitative techniques.

8.4.2.2 The cranial (L3 or L4) and caudal(pelvis) levels are secured using resin mounts and screws, with the L4–L5 or L5–L6 disc oriented in the horizontal position.

8.4.2.3 A marker is then secured to each vertebral level (L4 through L7) and oriented to permit detection by the motion analysis system.

8.4.2.4 To determine the multidirectional flexibility, six pure moments (flexion, extension, left and right lateral bending, and left and right axial rotation) are applied to the cranial end of the vertically oriented specimen while the caudal portion of the specimen (pelvis) remained fixed to a testing platform. A maximum applied pure moment of 0.27 Nm (**5**) is used for each loading mode and applied at a rate of 0.3 degrees/second.

8.4.2.5 A total of three load / unload cycles are performed for each motion with data analysis based on the final cycle.

8.4.2.6 For the six main motions (corresponding to the moments applied) the range of motion (ROM) and neutral zone (NZ) are calculated. ROM is defined as the peak displacement from the initial neutral position to maximum load, while NZ represents the motion from the initial neutral position to the unloaded position at the beginning of the third cycle.

8.4.2.7 To prevent desiccation during assessment, specimens are moistened with 0.9% NaCl sterile irrigation solution.

8.5 *Histology and Histopathological Analysis:*

8.5.1 *General*—Microscopic analysis may be used to qualitatively determine the presence of continuous normal bone formation from one transverse process to the next level of transverse process and confirm that the radiopaque materials as seen on radiographs are living bone. This type of analysis may also be used to evaluate the maturity (presence of more organized trabecular structures and a neocortex) and quality of the bone as well as cellular and tissue responses to the implant materials at each time point analyzed and over time, either qualitatively or semiquantitatively. However, there may be occasions when quantitative histomorphometric analysis is preferred to determine progressive bone graft material resorption and new bone formation over a period of time. The investigators should make their own assessments on whether a quantitative histomorphometric analysis is needed based on the implant material and the objective(s) of the study.

8.5.2 *Histology—Preparation of Explants and Slides:*

8.5.2.1 Spines should be explanted to include anatomical landmarks to enable accurate trimming and isolation of functional units.

8.5.2.2 Remove unnecessary muscle tissue from isolated units, taking care not to disturb the fusion masses.

8.5.2.3 Radiograph each specimen to produce a dorsal/ventral view.

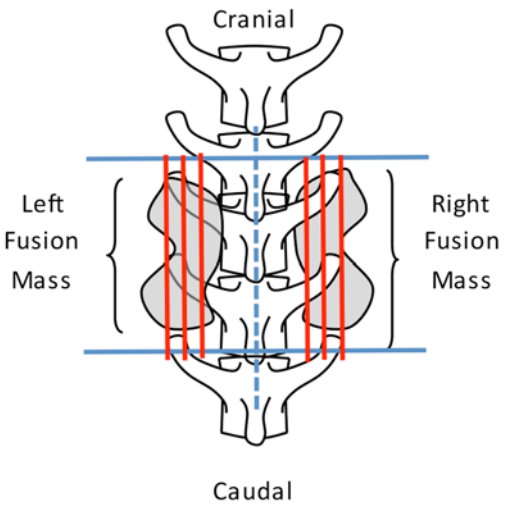
8.5.2.4 Trim specimens to isolate the treated level (see Fig. 8).

8.5.2.5 Half each specimen along the midline plane into left and right sides (see Fig. 8).

8.5.2.6 Embed each half in a medium that allows for retention of the implant material(s). Selection of embedding medium is dictated by implant material types. The decalcification process required to remove calcium from bone will also remove calcium from implant materials. Because of the decalcification process, all demineralized bone and new and old mineralized bone present in paraffin sections will stain similarly. Plastic such as methyl methacrylate embedding is recommended to enable visualization of implant materials containing demineralized bone, mineralized bone and synthetic bone materials to ensure the best possibility of differentiating between implant materials and newly-formed bone. In instances where it is believed that ground sections will not enable adequate evaluation of cellular responses to the implant materials, consider decalcification and paraffin embedding for one side to enable evaluation of cellular and tissue parameters and embedding the contralateral side in MMA for evaluation of implant material parameters.

8.5.2.7 Take pairs of thin sections or single ground sections from three locations spaced at least 300 microns from one another across the fusion mass that spans both decorticated transverse processes (TPs) and the fusion mass laying over the TPs, at the approximate locations shown in Fig. 8. The planes of section should be parallel to the midline cut. Actual locations will be dictated by the location of the fusion masses.

(1) Three planes of section are recommended to ensure full evaluation of each fusion mass as bone formation and implant resorption is often variable across the masses. Scientific justification demonstrating that fewer sections are representative of the whole fusion mass is recommended before sampling from fewer planes across each fusion mass. It is recommended



NOTE 1—The dashed blue line shows the midline trimming plane. The solid blue lines indicate the segment isolation trim planes. The red lines show the three sagittal planes of section across.

FIG. 8 Illustration of the Approximate Planes of Trimming and Sectioning for Histology

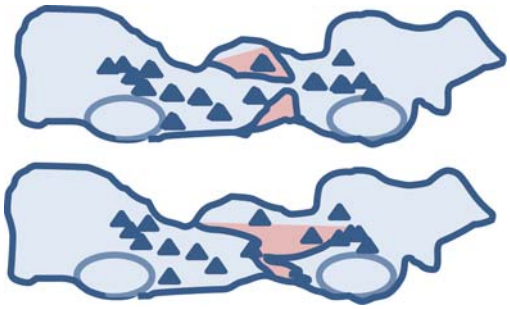
that the decision to reduce the number of sections sampled be based on preliminary data from all implant types to be tested in the study (test and controls).

(2) Selection of thin plastic versus ground plastic sections will depend on the material types implanted. Ground sections should be taken if the materials being tested crumbles from the tissue during cutting, removing bone and other soft tissue in the process of cutting, i.e., production of artifact that negatively impact interpretation. Thin sections can be used if soft tissue is not damaged and cutting artifact does not negatively impact interpretation or subsequent analyses.

8.5.2.8 Stain one of each pair of the thin sections with a bone stain such as Goldner's trichrome or other bone stain that enables evaluation of bone detail and sufficiently enables differentiation between bone and bone substitute implant materials. Stain the other of each pair with haematoxylin and eosin (H&E) stain to enable evaluation of cellular detail or stain ground sections with a bone stain such as Stevenel's blue and a counterstain such as a van Gieson stain to enable evaluation of cellular detail. Stain paraffin sections with H&E (not recommended if histomorphometric analysis is performed).

8.5.3 Semi-quantitative evaluation should be completed blind by a qualified Pathologist (board certified Anatomic Pathologist or PhD Experimental Pathologist) familiar with bone pathology and more specifically, the healing processes associated with implantation of biomaterials. Using plain and polarized light microscopy, histopathologic interpretation will be performed and will include review of all histology slides stained with H&E or other stains as appropriate to determine the presence of activated macrophages, giant cells, polymorphonuclear cells and general histiocytic infiltration according to ISO 10993-6 Annex E (see Appendix X1) and associated adverse responses such as fibrosis and necrosis.

8.5.3.1 Evaluate sections from all 3 levels for evidence of fusion. Assess each section for evidence of fusion (continuity of bone between transverse processes or, when TPs have been remodeled, across the entire fusion mass) by assigning a 1 to the section if evidence of fusion is present and 0 if not. Because of sampling (5 micron thin or 50-100 micron ground sections through the fusion masses) and assessment of multiple time points where the fusion process is more dynamic, sections may not always show bridging across the entire width of the fusion mass and may not always show fusion in every section taken from the fusion mass. Therefore, it is important to assess all slides taken. Also, discontinuity in calcified bone can occur because of the presence of cellular or fatty bone marrow or residual implant material at the bridge even in stable fusion masses. Therefore, both cellular and fatty bone marrow are considered part of bone for the determination of microscopically-evident fusion. If a calcified bone/bone marrow (cellular or fatty) bridge is evident between the TPs whether all the way across or in a small area such as shown in the top schematic of Fig. 9, assign a score of 1. Assign a score of 0 if fibrous tissue, fibrocartilage or cartilage prevents bridging with calcified bone/bone marrow that would enable stabilization of the fusion mass as shown in the bottom schematic of Fig. 9. Actual examples of fused and unfused masses are provided in Fig. 10.



NOTE 1—The ovals represent the TPs; the triangles represent residual implant material and the pink-colored area represents soft tissue at the bridge between the TPs. The shaded blue-grey area represents new bone and bone marrow. The top image represents a section that would be considered fused (scored 1) and the bottom represents a section that would be considered unfused (scored 0), due to the soft tissue plane cutting through the fusion mass and the clefts (dark blue lines) in the bone.

FIG. 9 Schematic View of a Sagittal Section Taken Through the Fusion Mass

8.5.3.2 Average scores for each specimen (including all sections from right and left halves) should be used for statistical analysis.

8.5.4 Evaluate sections from all three planes for tissue and cellular responses to the implanted materials. Scientific justification demonstrating that fewer sections are representative of the whole for all test and control implants being tested is recommended if fewer sections are to be selected for this analysis.

8.5.4.1 Evaluate sections for fusion maturation (lamellar bone vs woven), percent bone marrow (including fatty and cellular bone marrow), fibrosis, vascularity and fat associated with fibrosis, necrosis and inflammation (individual cell types) associated with healing and resorption using a semiquantitative scoring system according to the criteria provided in Appendix X1.

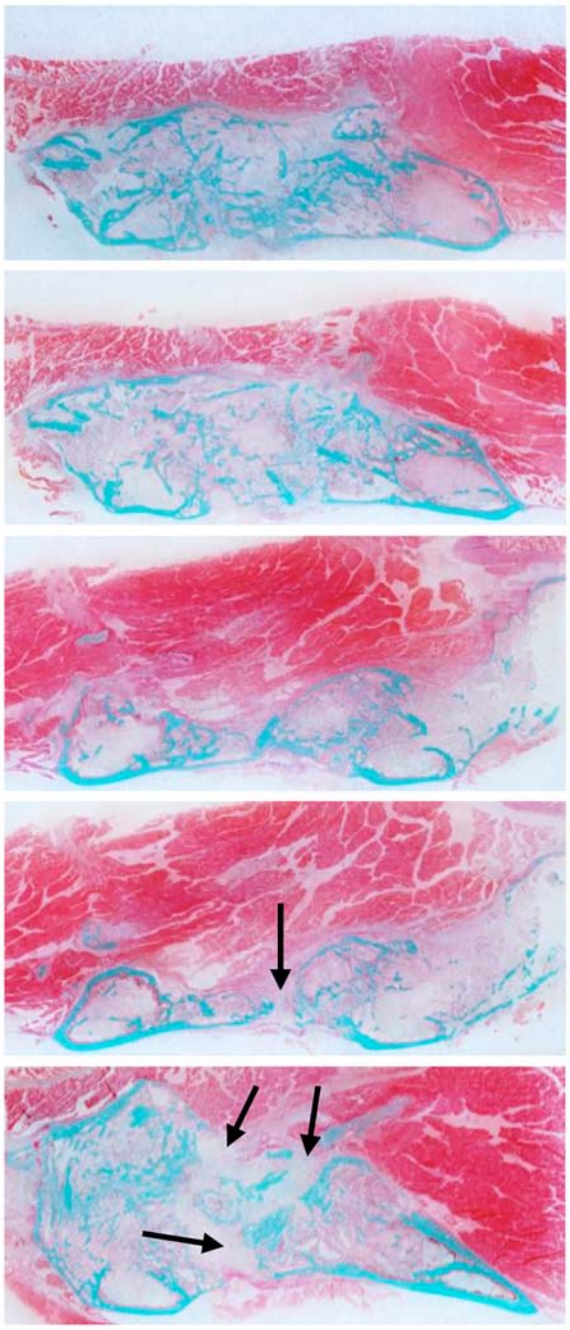
(1) If a quantitative (histomorphometry) assessment is not completed, evaluate percent residual implant material for test and control implant groups and percent calcified bone for all groups. Evaluate percent bone marrow for all groups. Semi-quantitative scores should be assessed as a function of the entire fusion mass.

8.5.4.2 Averaged scores for each specimen (including all sections from right and left halves) should be used for statistical analysis.

8.6 *Histomorphometric Analysis:*

8.6.1 Analyze slides (one or more slides selected for additional evaluation) for calcified bone and residual implant material. Evaluation should be completed by a qualified technician who has received training from a subject matter expert and is familiar with bone and biomaterials, as bone and synthetic biomaterials will take up bone stains which may make differentiation between bone and implant materials difficult.

8.6.1.1 In those cases in which color thresholds cannot be used to differentiate between new bone and implant material,



NOTE 1—(blue=bone, bright red=muscle, denser pink = marrow space and fatty marrow, light pink to white =fibrous tissue or cartilage). The TP boundaries are partially obliterated through initial decortication, and remodeling. The top section shows an obviously fused mass with obvious calcified bridging. The next section down shows some discontinuity across the mass, but with calcified bone/bone marrow bridging. The third image down shows a thin bone bridge, but substantial bone on either side of the bridge. All three top images would be considered fused (scored 1). The bottom two images show fibrous tissue or cartilage (arrows) between the TPs that prevents a bridge and would be considered unfused (scored 0) despite the substantial bone on either side. Goldner's trichrome stain, original magnification, 1×.

FIG. 10 View of Sagittal Sections Taken Through Fusion Massed Treated with Autograft

manual tracing may be required to separate and analyze the various tissues present within the fusion mass.

8.6.2 Capture scaled images of the region of interest (ROI). The entire fusion mass including the residual implant material, and the hard and soft tissue that make up the entire mass between the TPs and extending proximal and distal to the TPs as shown in the schematic) for histomorphometric analysis. Decorticated TPs may be remodeled, with less defined margins over time as the fusion mass matures. In such cases, the TPs may be included in the ROI across all the study groups and time points. The size of the fusion masses may also reduce with time as maturation occurs.

8.6.2.1 Images should be captured using a system that enables preparation of sufficiently high resolution images for

NOTE 1—The ovals represent the TPs, the triangles represent residual implant material, the pink-shaded area represents soft tissue and the blue-grey-shaded area represents new bone and bone marrow. The dark blue outer line is the whole ROI that encompasses all bone, soft tissue and residual implant material.

FIG. 11 Schematic View of a Sagittal Section Taken Through the Fusion Mass

analysis of bone and residual implant materials. The magnification selected will be dictated by the sensitivity of the image analysis system used but should be sufficiently high to ensure adequate capture of all parameters analyzed.

8.6.3 Using image analysis software such as ImagePro or Ostomeasure, select the ROI (the entire area occupied by the new bone, TPs, residual implant and the tissue reaction to the implant materials, new bone and bone marrow, residual implant and soft tissue associated with the implant). Measure the ROI area. Measure calcified bone area and residual implant area within the ROI. The area occupied by the transverse processes should be excluded from any histomorphometric analysis of new bone and calcified bone area at the implant site.

8.6.3.1 Calculate percent calcified bone and percent residual implant as a function of the total ROI area.

9. Statistical Analysis

9.1 All biomechanical data should be expressed as a ratio of the results from the fused to the results from the adjacent unfused level.

9.1.1 The means, standard error of the mean and confidence interval for the mean should be calculated at each time point.

9.2 All ordinal, semi-quantitative histopathological data should be considered for sections taken from both right and left fusion masses from a given animal.

9.2.1 For semiquantitative data, at a minimum, the median, mean, standard error of the mean and confidence interval for the mean should be calculated for each group at each time point.

9.2.2 A non-parametric test such as the Kruskal-Wallis or other appropriate test is recommended to compare multiple groups at each time point or a single group over multiple time points.

9.2.3 Specific comparisons between two groups or time points is recommended using a nonparametric test such as a Kolmogorov-Smirnov two sample, Wilcoxon rank-sum or other test, with appropriate adjustment for multiplicity.

9.3 All quantitative histomorphometric data should be averaged for sections taken from both right and left fusion masses.

9.3.1 For quantitative data, at a minimum, the means, standard error of the mean and confidence interval for the mean should be calculated for each group at each time point.

9.3.2 One or two-way analysis of variance (ANOVA) is recommended to determine differences between multiple groups or a single group over multiple time points. Differences should be verified between pairs of time points or groups using a Tukey range test or other appropriate test.

10. Criteria for Successful Fusion

10.1 For this model, it is expected that bone formation occurs between the transverse processes in sufficient amounts to bridge the transverse processes with sufficient mass to ensure stability of the fused level. At a minimum, evidence of fusion (by palpation) and implant resorption over time (by histology and/or histomorphometry), with a lack of evidence of adverse cellular responses to the implant materials (histopathological analysis), is required to demonstrate success of a bone graft intended to facilitate fusion. Additional supporting evidence from radiography (clinical and/or ex-vivo), histology (including histomorphometry), and biomechanical testing may be required to support a successful result.

10.2 Microscopic evidence of new bone formation between the transverse processes, in and of itself, will not constitute successful fusion unless the amount of new bone is substantive and bridges the transverse processes in a manner that is expected to carry load (i.e., may remodel or even reduce in mass, but matures with time) and prevent movement. Implant resorption is also expected to occur over time. A bone graft intended to facilitate fusion should not induce a cellular response that prevents bone formation and bone maturation.

11. Keywords

11.1 autograft; bone graft; fusion; intertransverse; posterolateral; rabbit; spine

APPENDIX

(Nonmandatory Information)

X1. GRADING SCALES

X1.1 See Table X1.1.

TABLE X1.1 Histopathologic Analysis Scoring System (Adapted from ISO 10993-6, Annex E)

Response	Score (*phf = per high powered (x400) field; ** Large infiltrate in one area or infiltrates in multiple areas; ***Larger numbers throughout the fusion mass)				
	0	1	2	3	4
Polymorphonuclear cells	0	Rare, 1-5/phf*	5-10/phf	Moderate infiltrate**	Extensive infiltrate***
Lymphocytes	0	Rare, 1-5/phf*	5-10/phf	Moderate infiltrate**	Extensive infiltrate***
Plasma cells	0	Rare, 1-5/phf*	5-10/phf	Moderate infiltrate**	Extensive infiltrate***
Macrophages	0	Rare, 1-5/phf*	5-10/phf	Moderate infiltrate**	Extensive infiltrate***
Giant cells	0	Rare, 1-5/phf*	3-5/phf	Moderate infiltrate**	Extensive infiltrate***
Necrosis	0	Very small amount: Tissue or cell necrosis occupies a very small focal area	Small amount: Tissue or cell necrosis occupies only a small area at the periphery or within the fusion mass	Moderate amount: necrosis occupies a portion of the area at the periphery or within the fusion mass	Extensive: necrosis throughout the implant area and/or adjacent to the implant
Fibrosis	0	Minimal amount: One or 2 narrow or short bands	Small amount: narrow band or bands of fibrous tissue	Moderate: Thick band or many bands of fibrous tissue	Extensive amount: Little tissue other than fibrous tissue within the implant area
Neovascularisation associated with fibrosis	0	Minimal capillary proliferation focal, 1-3 buds	Groups of 4-7 capillaries with supporting fibroblastic structures	Broad band of capillaries with supporting structures	Extensive band of capillaries with supporting fibroblastic structures
Fatty infiltrate associated with fibrosis	0	Minimal amount of fat associated with fibrosis	Several layers of fat and fibrosis	Elongated and broad accumulation of fat cells about the implant site	Extensive fat completely surrounding the implant
Residual Implant	0	Occupies<1% of defect area	Occupies 1-10% of defect area	Occupies 11-25% of defect area	Occupies >25% of the defect area
New Calcified Bone	0	Occupies 10-25% of implant area	Occupies 26-50% of implant area	Occupies 51-75% of implant area	Occupies 76-100% of implant area
New Bone Marrow (fatty + cellular)	0	Occupies 10-25% of implant area	Occupies 26-50% of implant area	Occupies 51-75% of implant area	Occupies 76-100% of implant area

REFERENCES

(1) Boden, S.D., Schimandle, J.H., Hutton, W.C., "An Experimental Lumbar Intertransverse Process Spinal Fusion Model. Radiographic, Histologic, and Biomechanical Healing Characteristics." *Spine*, 4 (20), pp 412-420, 1995.

(2) Riordan, A.M., Rangarajan, R., Balts, J.W., Hsu, W.K., Anderson, P.A. Reliability of the Rabbit Postero-Lateral Spinal Fusion Model: A Meta-Analysis. *JOR*. 31 (8), pp 1261-1269, 2013.

(3) Palumbo, M., Valdes, M., Robertson, A., Sheikh, S., Lucas, P., "Posterolateral intertransverse lumbar arthrodesis in the New Zealand white rabbit model: I. Surgical anatomy." *Spine J*. Vol. 4, pp 287-292, 2004.

(4) Valdes, M., Palumbo, M., Appel, J., McAllister, S., Ehrlich, M., "Posterolateral intertransverse lumbar arthrodesis in the New Zealand White rabbit model: I. Operative technique." *Spine J*. Vol. 4, pp 293-299, 2004.

(5) Grauer, J., Erulkar, J., Patel, T., Pamjabi, M., "Biomechanical evaluation of the New Zealand white rabbit lumbar spine: a physiologic characterization." *Eur Spine J*, 9, pp 250-255, 2000.