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

This standard is issued under the fixed designation F2884; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

## 1. Scope

1.1 This guide covers general guidelines for the pre-clinical *in vivo* assessment of tissue-engineered medical products (TEMPs) intended to repair or regenerate bone in an interbody and/or posterolateral spinal environment. TEMPs included in this guide may be composed of, but are not limited to, natural or synthetic biomaterials or composites thereof, and may contain cells or biologically active agents such as growth factors, synthetic peptides, plasmids, or cDNA. The models described in this document represent a stringent test of a material's ability to induce and/or augment bone growth in the spinal environment.

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

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

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

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

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

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

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

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

## 2. Referenced Documents

2.1 *ASTM Standards:*[2]

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

F565 Practice for Care and Handling of Orthopedic Implants and Instruments

F895 Test Method for Agar Diffusion Cell Culture Screening for Cytotoxicity

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

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

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

2.2 *Other Standards:*

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

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

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[2] For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

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

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

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

## 3. Terminology

3.1 *Definitions:*

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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