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

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1. Scope

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

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

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

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

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

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

1.7 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety, health, and environmental practices and determine the applicability of regulatory limitations prior to use.*

1.8 *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 Documents:*

21 CFR Part 58 Good Laboratory Practice for Nonclinical Laboratory Studies[3]

21 CFR 610.12 General Biological Products Standards—Sterility[3]

3. Terminology

3.1 *Definitions:*

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

4. Significance and Use

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

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

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

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

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

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

5. Animal Models

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

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

5.1 *Defect Size:*

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