



Designation: F2451 – 05

Standard Guide for *in vivo* Assessment of Implantable Devices Intended to Repair or Regenerate Articular Cartilage¹

This standard is issued under the fixed designation F2451; 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 *in vivo* assessment of implantable devices intended to repair or regenerate articular cartilage. Devices 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.

1.2 Guidelines include a description and rationale of various animal models utilizing a range of species such as rabbit (lupine), dog (canine), pig (porcine), goat (caprine), sheep (ovine), and horse (equine). Outcome measures based on histologic, biochemical, and mechanical analyses are briefly described 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 product. ASTM standards for these steps are available in Reference Documents.

1.4 The values stated in SI units are to be regarded as the standard.

1.5 *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 and health practices and determine the applicability of regulatory requirements prior to use.*

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 Documents:

ISO-10993 Biological Evaluation of Medical Devices—Part 5: Tests for *in vitro* Cytotoxicity³

21 CFR Part 58 Good Laboratory Practice for Nonclinical Laboratory Studies⁴

3. Terminology

3.1 Definitions:

3.1.1 *cartilage regeneration*—the formation of articular-like cartilage that has histologic, biochemical, and mechanical properties similar to that of native articular cartilage (**1, 2**).⁵

3.1.2 *cartilage repair*—the process of healing injured cartilage or its replacement through cell proliferation and synthesis of new extracellular matrix (**1, 2**).

3.1.3 *compact bone*—classification of ossified bony connective tissue characterized by the presence of osteons containing lamellar bone.

3.1.4 *femoral condyles*—the anatomic site corresponding to the distal end of the femur characterized by medial and lateral convex surfaces that are lined by cartilage and articulate with the proximal tibia and medial and lateral menisci.

3.1.5 *fibrocartilage*—disorganized cartilaginous tissue having an abnormally high content of type I collagen.

3.1.6 *growth plate*—the anatomic location within the epiphyseal region of long bones corresponding to the site of

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.

Current edition approved April 1, 2005. Published May 2005. DOI: 10.1520/F2451-05.

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.

⁴ Available from U.S. Government Printing Office Superintendent of Documents, 732 N. Capitol St., NW, Mail Stop: SDE, Washington, DC 20401.

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

growth of bone through endochondral bone formation. The growth plate in skeletally mature animals is fused.

3.1.7 *hyaline articular cartilage*—cartilagenous connective tissue located in diarthrodial joints and characterized by its localization to articulating surfaces.

3.1.8 *marrow*—also called *myeloid tissue*; soft, gelatinous tissue that fills the cavities of the bones. It is either red or yellow, depending upon the preponderance of vascular (red) or fatty (yellow) tissue.

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

3.1.10 *patella*—the bone of the knee joint which articulates within the trochlear groove of the femur.

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

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

3.1.13 *subchondral plate*—the margin of compact bone in direct apposition to the articular cartilage.

3.1.14 *synovial fluid*—the fluid secreted by synovium providing lubrication and nutrition to the joint surfaces.

3.1.15 *synovium*—the epithelial lining of synovial joint cavities that produce synovial fluid.

3.1.16 *tidemark*—the anatomic site in articular cartilage corresponding to the margin between cartilage and the underlying calcified cartilage.

3.1.17 *trabecular bone*—classification of ossified boney connective tissue characterized by spicules surrounded by marrow space.

3.1.18 *trochlear groove*—the anatomic site on the distal end of the femur corresponding to the region of articulation with the patella.

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 intended for the clinical repair or regeneration of articular cartilage.

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 or devices, 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 IDE (Investigational Device Exemption), PMA (Premarket Approval), or 510K submissions should conform to appropriate FDA guidelines for development of medical devices.

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 cartilage defect size and location.

5.1 Joint Size and Load:

5.1.1 A high proportion of hyaline cartilage injuries in humans occur in the knee joint predominantly in the medial compartment (that is, medial femoral condyle and tibial plateau). Accordingly, the knee joint is commonly used for assessing cartilage repair/regeneration in animal models.

5.1.2 The knee is a complex diarthrodial joint involving primarily two separate articulations; femoropatellar and femorotibial. The articular surfaces of the distal femur and proximal tibia are incongruent and contain wedge shaped fibrocartilagenous menisci separating the articular surfaces. Contact between the cartilage of the femoral condyles and that of the tibial plateau occurs at the innermost central region of each medial and lateral meniscus. Mechanical load is distributed directly from the femur to the tibia as well as indirectly through the menisci. The patella articulates with the femoral condyle within the trochlear groove.

5.1.3 Significant variability exists between animal species with respect to the weight of the animal, joint anatomy, and gait thereby influencing joint kinetics, range of motion, and mechanical forces on joint surfaces. These factors influence the thickness and distribution of articular cartilage within the joints as well as macromolecular content, distribution, and collagen architecture. These factors play a significant role in the response to injury or disease of articular cartilage (see [Table 1](#)). The user should consider carefully the animal model that is appropriate for the stage of investigation of an implanted device ([3](#)).

TABLE 1 Animal Models for the Assessment of Cartilage Repair

Species	Breed Commonly Used	Age of Adult Equivalency	Weight at Adult Equivalency	Defect Sites Commonly Used	Cartilage Thickness at Femoral Condyle (mm)	Critical Size Defect (Diameter in mm)
Rabbit ^A (Lupus or Lupine)	New Zealand White	9 months	3–4 kg	FC, TG, TP, P	0.25–0.75	3
Dog ^B (Canine)	Mongrel, Beagle	>1–2 years	15–30 kg	FC, TG, P	1.3	—
Pig ^B (Porcine)	Minipig	10 months–1 year	20–40 kg	FC, TG	—	—
Goat ^B (Caprine)	Spanish, Dairy, Boer Cross	2–3 years	40–70 kg	FC, TG, TP, P	1.5–2	—
Sheep ^B (Ovine)	Suffolk or Texel	2–3 years	35–80 kg	FC, TG	1.7	7
Horse ^B (Equine)	Mixed, Thoroughbred, Quarter Horse	2–4 years	400–500 kg	FC, TG, RC	2–3	9

^A small animal.

^B large animal; FC, femoral condyle; TG, trochlear groove; TP, tibial plateau; P, Patella; RC, radial carpal.

5.1.4 Mechanical load has been shown to affect cartilage repair. Amongst the mechanobiological factors, the intermittent hydrostatic pressure and shear stresses play an important role in modulating cartilage development, and maintenance as well as cartilage degeneration (4, 5). The impact of mechanical load extent or duration on the implanted device, surrounding native articular cartilage, and underlying bone varies depending on the anatomic site and the position of the joint (6). The defect site chosen to evaluate implants should, therefore, factor the impact of mechanical load on the performance of the implant.

5.1.5 It is suggested that the gait and stance of a particular species be considered when factoring the extent of exposure of the implant site to stress during standing and motion.

5.1.6 The extent of compressive and shear forces in the femoral condyles, trochlear groove, and tibial plateau differ significantly as do differing anatomic sites of the same articular surface.

5.1.7 It is recommended that an appropriate species and anatomic site be chosen having articular surfaces and thickness sufficiently large to adequately investigate and optimize the formulation, design, dimensions, and associated instrumentation envisaged for human use.

5.1.8 Larger animals are more appropriate for studying repair in joints that have greater articular cartilage surface areas and a thickness that more closely approximates that of humans.

5.1.9 Larger defect dimensions generally require a method of fixation to secure the implant and thereby reduce implant dislocation. The method of implant immobilization can negatively impact both the surrounding host tissue and repair tissue. Accordingly, the difference in the design of the test device in small animals 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 requiring fixation.

5.1.10 For each species, a critical size defect is defined as the minimum defect dimension (in diameter) that the animal is incapable of repairing without intervention. The diameter of critical defects generally differ for each species and should be considered carefully when designing the implant dimensions and method of fixation.

5.2 Handling:

5.2.1 Exposure of implants to extreme and highly variable mechanical forces as a result of jumping, running, hyperextension, or hyperflexion of the joint can lead to increased variability in outcome measures.

5.2.2 Care should be used to reduce stress or other factors that cause behaviors associated with rapid or extreme, or both, movements of joints.

5.3 Gender:

5.3.1 Due to the impact of circulating steroids on cartilage and bone metabolism and regeneration, the choice of gender should be considered. Animals in lactation should not be used.

5.3.2 It is recommended that the gender be the same within the cohort.

5.4 Age:

5.4.1 Bone and cartilage undergo dynamic changes in metabolism and remodeling during growth. Due to the impact of these physiologic processes on tissue repair, the age of a

particular species should be chosen to exceed the age of skeletal maturity. The cohorts should have fused epiphyseal growth plates. Skeletal maturity varies between species and can be generally determined radiographically if necessary.

5.4.2 Older animals have a higher propensity for osteopenia and degenerative joint diseases such as osteoarthritis, and have a decreased capacity to repair articular cartilage defects. If specific conditions are considered important for the intended device 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 decreases with age (7). Thus, reparative processes that are dependent on the number and activity of native cells may be partially compromised in older animals.

5.5 Study Duration:

5.5.1 The length of the study depends on the stage of device development, the species used, the size of the defect, and composition and design of implant.

5.5.2 In small animals, small defects implanted for 6 to 8 weeks provide information regarding residence time of implant and fixation device as well as the type of repair.

5.5.3 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.

5.5.4 Periods of 6 to 12 months are generally necessary to gain confidence in the extent of success in the repair or regeneration of articular cartilage based on histologic and biochemical outcome measures, including the interface with adjacent cartilage and subchondral bone, as well as the opposing articular surface.

5.6 *Rabbit Model*—The femoral condyle and trochlear groove are most frequently used as sites for evaluation of implants in rabbits (8-14). The use of the patella has been investigated as well (15, 16).

5.6.1 The use of rabbits is generally more economical compared to larger species.

5.6.2 Due to the small surface area and thickness of cartilage in rabbits, the dimensions of the defect are limited.

5.6.3 Evaluation of methods of device fixation in the defect is less feasible in the rabbit model. Accordingly, the rabbit model is best suited for assessing biocompatibility, material formulations, and basic device design screening.

5.6.4 In general, the rate, type, and extent of repair of rabbit articular cartilage is greater than that of larger animal models and may be related to higher metabolic activity and density of pluripotent stem cells near the defect site.

5.7 *Dog Model*—The trochlear groove and femoral condyle are used as sites for evaluation of implants in dog (17-19).

5.7.1 Joint surfaces in the knees of dogs are considered intermediate in size between that of rabbit and that of adult goat, sheep, and horse.

5.8 *Pig Model*—The femoral condyle has been used for evaluation of implants in the pig model (20, 21).

5.8.1 The anatomy of the tibio-femoral joint angle of pigs differs from many other quadrupeds in that it is reduced in its range of motion.