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# Standard Guide for *in vivo* Assessment of Implantable Devices Intended to Repair or Regenerate Articular Cartilage[1]

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 standard. No other units of measurement are included in this 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* Cytotoxicity[3]

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

## 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 boney 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.

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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.

[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.

3.1.5 *fibrocartilage*—disorganized cartilagenous 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 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 extracelluar 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

**TABLE 1 Animal Models for the Assessment of Cartilage Repair**

| Species | Breed Commonly Used | Age of Adult Eqivalancy | Weight at Adult Equivalancy | 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.

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**).

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.

5.9 *Sheep Model*—The femoral condyle is commonly used as the test site for implants in sheep (**22-24**).

5.9.1 Contact between the femoral condyles and tibial plateau occurs caudally on the tibial plateau throughout the normal range of motion. The femorotibial articulation moves from 72 ± 3° in full flexion to 145 ± 5° in full extension (**25**).

5.9.2 Tissue calcification has been observed postoperatively in some studies using sheep (**22, 26**).

5.10 *Goat Model*—The femoral condyle and trochlear groove are most frequently used as the implant test site in goat (**27-30**). However, the use of other surfaces such as the tibial plateau (**31**) and patella (**29**) has been reported.

5.10.1 In comparison to sheep, goats are generally less adverse to human interaction and are therefore easier to handle.

5.10.2 Goats should be screened by blood test for caprine encephalitis prior to inclusion in cohort group.

5.10.3 Contact between the femoral condyles and tibial plateau occurs caudally on the tibial plateau throughout the normal range of motion. The femorotibial articulation range is similar to that of sheep.

5.10.4 Due largely to their stifle size, cartilagenous thickness, availability, and ease of handling, goats represent a favorable animal model for cartilage repair studies (**27, 28, 32-35**).

5.11 *Horse Model*—The femoral lateral trochlear ridge and condyle are most frequently used as the test site in the horse model, however, reports using the radial carpal bone in the mid-carpal (intercarpal) joint exist as well. The lateral trochlear ridge is relatively flat compared to the medial trochlear ridge and forms an excellent site to make multiple large defects (>15 mm). The condyle is more typically curved as for other species. Large defects can be made at both sites (critical size defect somewhere between 3 and 9 mm (**36**)). Only species routinely used for arthroscopic implantation of experimental devices; arthrotomy is satisfactory alternative.

5.11.1 *Femoral Lateral Trochlear Ridge* (**37-40**)—Have a significant cartilage surface area with the capacity for larger diameter defects. Cartilage thickness is comparable to humans.

5.11.2 *Femoral Condyle* (**36, 41**)—Large surface area, direct weight bearing, cartilage thickness comparable to human.

5.11.3 *Radial Carpal Bone* (**42, 43**)—Radial carpal bone of midcarpal joint of forelimb differs significantly from knee joint (more akin to the wrist) in that it lacks a meniscus and patella.

5.11.4 Despite the similarity in cartilage thickness to humans, higher costs associated with purchase, husbandry, and surgery have limited the use of horse models. However, arthroscopic techniques and the amount of retrieved tissue for analysis make it attractive.

## 6. Considerations for Defect Site

6.1 *Femoral Condyle:*

6.1.1 Depending on the size of the animal, the dimensions of a defect to be used for implants can vary widely from 2 to 15 mm in diameter and 1 to 10 mm in depth. Generally, the size of defect should not exceed approximately 15 to 20 % of the articulating surface or 50 to 60 % of the condylar width.

6.1.2 Due to the convex curvature of the femoral condyle, the depth of the defect can differ from the center to the margins.

6.1.3 Depending on the location of the defect on the femoral condyle, the impact of articulation with both the meniscus and the tibial plateau should be considered, particularly in the resting position.

6.2 *Trochlear Groove:*

6.2.1 The trochlear groove can be used to evaluate a site that is subjected to a load and shear that is different than that of the femoral condyle of the same animal.

6.2.2 To gain access to this site, the patella may be dislocated (luxated) prior to defect generation.

6.2.3 The thickness of cartilage in the trochlear groove is generally less than that of the femoral condyle in the same animal and should be factored into prototype device design.

6.2.4 Due to the concave nature of the trochlear groove, the depth of the defect may vary depending on the defect dimensions and the location within the groove (wall versus base).

6.2.5 Consideration should be given to the mechanical load differences that exist between rostral and caudal (proximal and distal) sites on the articular surface of the trochlear groove, and the effect these differences have on repair.

6.2.6 Horse is unusual in that the trochlear groove is asymmetrical; lateral trochlear ridge is flat, presenting better area for evaluation, without the need to luxate the patella and without thickness variation.

6.3 *Tibial Plateau:*

6.3.1 The tibial plateau has been used by some investigators. Difficulty in surgical access to this articular surface due to the femoral condyle, meniscus, and cruciate ligaments make it less frequently used compared to the femoral condyles and trochlear groove.

## 7. Considerations for Defect Type, Implant Fixation, and Joint Immobilization

7.1 *Chondral and Osteochondral Defects:*

7.1.1 A chondral (full or partial-thickness) or osteochondral (full-thickness and trans-osseous) defect can be created using appropriate tools to achieve consistent removal of cartilage and bone without excessive damage to surrounding tissues.

7.1.2 For chondral defects a trephine or drill may be used with care to avoid removal or damage to underlying bone. Caution should be used in choosing the speed of the drill and the amount of pressure applied due to the potential generation of excessive heat that can cause thermal necrosis to surrounding tissues. Alternatively, curettage may be used to remove cartilage to the level of the subchondral bone. Uniform lesions may be outlined with biopsy punches, available in many sizes, and the cartilage then removed with small bone curettes.

7.1.3 It should be noted that variability exists in both the depth of cartilage and the thickness of the subchondral plate. The user should factor this variability into deciding on the depth to achieve a consistent chondral or osteochondral defect.

7.1.4 Care should be taken to create a defect that is perpendicular to the articular surface.

7.1.5 Multiple defects can be created on an articular surface or within the same joint (**44**) to enable evaluation of more than one implant. However, the size and location of multiple defects and the effect of such on surrounding tissues should be considered. Negative controls and other controls should be included.

7.1.6 Excessive cartilage damage can increase the propensity for chronic synovitis.

7.1.7 Unstable mechanical load distribution due to excessively large or multiple defects in the same joint can compromise the surrounding articular cartilage as well as assessment of device performance.

7.2 *Microfracture:*

7.2.1 In cases where access to marrow containing cells is desired, microfracture of the subchondral bone at the base of the defect can be used (**45**).

7.2.2 Appropriate instrumentation should be used to produce consistent sites of bone penetration and minimize excessive damage to the subchondral plate.

7.2.3 The response of subchondral bone to microfracture can include variable levels of resorption (osteolysis). Factors believed to influence osteolysis includes extent of bone damage, mechanical load, synovial fluid, and degradation products of implanted materials.

7.3 *Implant Fixation:*

7.3.1 Depending on the size and location of the defect and the species used, an appropriate method of implant immobilization will be necessary to prevent excessive movement or dislocation, or both.

7.3.2 The fixation method should reflect the extent of stress that will be experienced during standing and motion.

7.3.3 The short and long-term impact of the fixation method on the surrounding tissue and on the performance of the implanted device should be considered.

7.4 *Joint Loading and Immobilization:*

7.4.1 The animal joint anatomy and joint size as well as gait should be taken into account to determine the appropriate immobilization modality.

7.4.2 The use of splints, external fixators, and casts can be used to reduce joint motion and loading for variable periods following surgery. There should be a point when the joint is restored to normal activity and unrestricted motion for an appropriate amount of time.

7.4.3 The impact of disuse atrophy and potentially negative consequences to the cartilage should be considered when choosing period of immobilization.

7.4.4 Continuous passive motion has been shown to provide some level of benefit to regenerative process following cartilage injury in humans and animals (**8**). Implementation of similar therapeutic modalities in animal models is less feasible and has not been widely accepted.

7.4.5 The impact of limited access to surgical incision sites associated with the use of casts and splints should be factored into the postoperative care regime. The horse also initiates load bearing immediately following surgery, and some sites such as femoral condyle and trochlear groove can not be protected from load after surgery. A qualified veterinarian should examine animals routinely for any gross abnormalities and signs of excessive discomfort associated with joint immobilization strategies.

## 8. Test Procedures

8.1 *Implant Preparation:*

8.1.1 All materials to be implanted into animals should be verified to be noncytotoxic and biocompatible. Implant components can be sterilized and prepared aseptically or end-point sterilized by methods known to be acceptable to the implant composition and function.

8.1.2 Bioburden testing should be completed on representative test articles.

8.1.3 See Guide F2150, Practices F1983, F981, F565, and Test Method F895. Practice F1983 covers the assessment of compatibility of absorbable biomaterials for implant applications.

8.2 *Defect Generation:*

8.2.1 The joint and synovial fluid should be examined for evidence of unacceptable pathology. The size of the defects should be standard and uniform.

8.2.2 Defects should be irrigated both during and following drilling to reduce heat and remove residual particulate bone and cartilage prior to device implantation.

8.2.3 To reduce damage to tissues due to thermonecrosis, a hand held rotary drill or motorized drill adjusted to a speed not exceeding 500 rpm should be used.

8.2.4 The drill bit design should reduce the potential travel during drilling. A trochar designed to penetrate the articular cartilage layer can also be used to center the drill and reduce eccentric movement during drilling.

8.2.5 The removal of cartilage to the level of subchondral plate to generate a chondral defect should not elicit bleeding. Penetration of the subchondral plate to generate an osteochondral defect will generally result in punctate bleeding from the bony surface depending on depth.

8.2.6 It is recommended that heavy bleeding be controlled using sterile swabs or hemostatic sponge before the introduction of the test device. The extent of bleeding is highly variable between species and cohorts.

8.2.7 During the surgical procedure, the joint surface should be routinely moistened with sterile buffered saline to prevent dehydration.

8.3 *Test Device Implantation and Fixation:*

8.3.1 The test device should be implanted in a standard and reproducible manner.

8.3.2 Care should be exercised to ensure that the surrounding cartilage is not excessively damaged and that the device is in contact with the vertical walls of the defect at the margins and the bone at the base (for full thickness defects).

8.3.3 If a tissue flap (that is, periosteal membrane) is to be used, it should be anchored to the native cartilage in a manner that minimizes damage to the adjacent and opposing articulating cartilage.

8.3.4 The device should be placed at a depth that results in an articular surface that is adequately matched (flush) with level of the surrounding articular surface.

8.3.5 Suture closure of the synovial cavity should attempt to minimize exposure of articular surfaces to suture abrasion during the in life period. Standard fascial, muscular, and skin incision site closure can be performed with sutures and staples.

8.4 *Recovery and Husbandry:*

8.4.1 Recovery conditions should be designed to reduce potential for stress and excessive motion. For goats, sheep, and horses recovery pens that are sized to reduce excessive range of mobility for a period of two to three days are recommended.

8.4.2 All housing conditions should be USDA approved.

8.4.3 Animals should be monitored frequently and observations recorded to ascertain appropriate health and physical condition.

8.4.4 A veterinarian should approve the health condition of animals prior to returning them to larger groups or herds.

8.5 *In Life Period:*

8.5.1 The use of splints versus standard dressings can reduce joint motion and loading, however, the impact of disuse atrophy and potentially negative consequence to the cartilage should be considered when choosing length of treatment.

8.5.2 Radiographs should be used as appropriate for a given study to assess placement of the implants.

8.5.3 Following recovery, large animals should be contained within protected stalls for a minimum of nine days. After this period the animals can either remain in protected stalls or allowed to roam freely in group herds.

8.5.4 A qualified veterinarian should examine animals routinely for any gross abnormalities and for signs of discomfort.

8.6 *Necropsy:*

8.6.1 Animals should be euthanized in a humane manner according to accepted practices of the Animal Welfare Act.

8.6.2 Necropsy should be performed to determine if there are any gross abnormalities within the joint that may affect the outcome of the study. Practice F561 should be used to obtain specimens at necropsy. In addition, to those procedures described in Practice F561, gross evaluation should include: *(1)* a description of the color and quality of synovial fluid and appearance of the joint cavity lining, *(2)* the appearance of the native cartilage surfaces (presence or absence of fibrillation's), *(3)* appearance of the surrounding bone (presence or absence of osteophytes), *(4)* a description of the color and quality of the repair site tissue (including surface appearance and texture, and recreation of contour), and extent of integration of the implant.

8.6.3 The implanted site should be removed along with the surrounding cartilage and bone.

8.6.4 Articulating surfaces directly opposed to the implantation site may also be harvested along with a standardized amount of the surrounding cartilage and underlying bone.

8.6.5 Retrieved tissue should be placed in a solution consistent with intended outcome measures such as histology (decalcified paraffin versus nondecalcified plastic embedded), biochemistry, or mechanical testing.

8.6.6 Synovial tissue from several standard sites should also be harvested for evaluation of particle uptake and if possible, cellular recruitment to accumulated particles.

## 9. Evaluation and Results

9.1 *Histology*—For histologic processing procedures, refer to Practice F561. Histological sections should be used to assess the amount and quality of tissue regeneration or repair within the defect. Histologic sections should be serially cut and stained in a manner to allow for assessment of the quality of tissue and for detection of glycosaminoglycans. Standard stains include: Weigarts, Hemotoxylin and Eosin, Safranin-O, Toluidine Blue, analine blue or Modified Trichrome stain, or both(**46-50**).

9.1.1 *Microscopic Analysis and Scoring:*

9.1.1.1 Histologic sections should be analyzed for adverse tissue reactions using standard histopathologic indices.

9.1.1.2 For assessment of device performance, a scoring system such as that of O'Driscoll (**10**) should be utilized to determine the following:

*(1)* Tissue quality (hyaline versus fibrocartilage) within and surrounding the defect site,

*(2)* Surface appearance and extent of continuity with native cartilage,

*(3)* Extent of integration with native bone and cartilage,

*(4)* Quality of subchondral bone reconstitution,

*(5)* Cell morphology, and

*(6)* Quality of tissue surrounding fixation device.

9.1.1.3 Histomorphometric analyses can be utilized to measure histologic parameters such as thickness, integration, cell number, and surface quality.

9.1.1.4 Time points of less than six months do not necessarily reflect the long-term outcome due to the potential for changes in the biochemical composition and organization of repair tissue over time.

9.1.1.5 Short-term histologic evaluation can be used for screening and optimization while long-term assessment should be based on histologic, biochemical, and possibly mechanical measures.

9.2 *Biochemistry*—Normal hyaline articular cartilage consists primarily of Type II collagen and proteoglycans. Biochemical quantification of proteins and proteoglycans in repair tissue compared to native cartilage can provide useful information regarding the extent and quality of repair.