Review of Animal Models of Osteoarthritis

23 October 2016

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SCIENCE FOR LIFE-CHANGING IMPACT

Overview

- Animal models of osteoarthritis (OA) are widely used to study the pathogenesis and progression of osteoarthritis and to evaluate potential disease modifying agents (DMA) or pain control modalities to treat OA in human and animal patients.
- No animal model perfectly duplicates the onset and progression of OA in humans.
- Each animal model has assets and limitations which much be thoroughly understood in order to appropriately use and interpret the data generated.

Broad categories of OA in humans

- Spontaneous (age-related)
- Traumatic
- Congenital/inherited

Modalities to assess human OA

- Physical assessment/questionnaires
  - WOMAC (pain, stiffness, function)
- Imaging
  - Radiography
    - K-L scale (joint space narrowing, osteophytes, sclerosis)
  - MRI, PET
- Biomarkers (CTxII, COMP, etc)
- Histopathology of joint fragments collected during joint replacement (Mankin, OACH)

Conundrums in Human OA

- Degree of pain and/or dysfunction may have little or no correlation with radiologic findings
  - Some patients have significant pain but not major changes in joint structures and vice versa
  - MRI findings of bone marrow lesions and synovitis have better correlations with pain
- Variable Progression (slow vs. rapid)
- Spontaneous OA often not presented until quite advanced.

Characteristics of an ideal animal model for OA

- Similar joint structure and physiology to human
- Similar joint biomechanics to human
- Skeletal maturity
- Consistent, predictable responses to injury
- Reproducible assessments
- More rapid progression (weeks to months)
- Ease of handling

Pros and cons of models of OA in different species.

Table 1. Pros and cons of models of OA in different species

Species	Advantages	Disadvantages	Skeletal maturity	References
Mouse	Low cost: easy management and handling Transgenic lines available Genome sequenced Small amount of drugs required in interventional studies	Thin cartilage without clear zonal structure (compared with human cartilage) Small size, limiting technical procedures such as arthroscopy	10 weeks	Fang and Beier, 2014; Glasson et al., 2010
Rat	Low cost: easy management and handling Thick cartilage with complex zonal structure comparable to human cartilage Transgenic animals available in the near future Genome sequenced Availability of pain models	More expensive than mice Small size, limiting technical procedures such as arthroscopy	3 months	Gerwin et al., 2010
Guinea pig	Easy management and handling Prone to spontaneous osteoarthritis	More expensive than mice No transgenic lines available Sedentary lifestyle Small size, limiting technical procedures such as arthroscopy	4-6 months	Kraus et al., 2010
Rabbit	Easy management and handling Size allows easy tissue and fluid collection, and analysis of surgical strategies	More expensive than mice No transgenic lines available Spontaneous healing of cartilage lesions	8-9 months	Laverty et al., 2010
Dog	Tractable and trainable Size allows easy tissue and fluid collection, and analysis of surgical strategies Some strains develop spontaneous OA	High costs No transgenic lines available Genetic and phenotypic variability Public perception	9-18 months	Cook et al., 2010
Goat/sheep	Relatively easy management and handling Size allows easy tissue and fluid collection, and analysis of surgical strategies Thick cartilage and larger joints	High costs No transgenic lines available Genetic and phenotypic variability Ruminant digestive system can interfere with oral drugs	2 years	Little et al., 2010
Horse	Size allows easy tissue and fluid collection, and analysis of surgical strategies Thick cartilage and larger joints Develops spontaneous OA	High costs No transgenic lines available Genetic and phenotypic variability Public perception	2 years	McIlwraith et al., 2010

Sarah Thysen et al. Dis. Model. Mech. 2015;8:17-30

Reality check

- There are no perfect animal models for either spontaneous or traumatic OA in humans
- Choose the animal model that will best allow your hypothesis to be tested.

Sources of Variability in Animal Models

- Species sources of variability
  - Joint anatomy
  - Biomechanics
  - Responses
    - To injury
    - To therapies
    - Over time
- Most assessments are subjective
  - Variability between labs, assessors
- Variable study designs

Articular Cartilage Anatomy, Cynomolgus Monkey

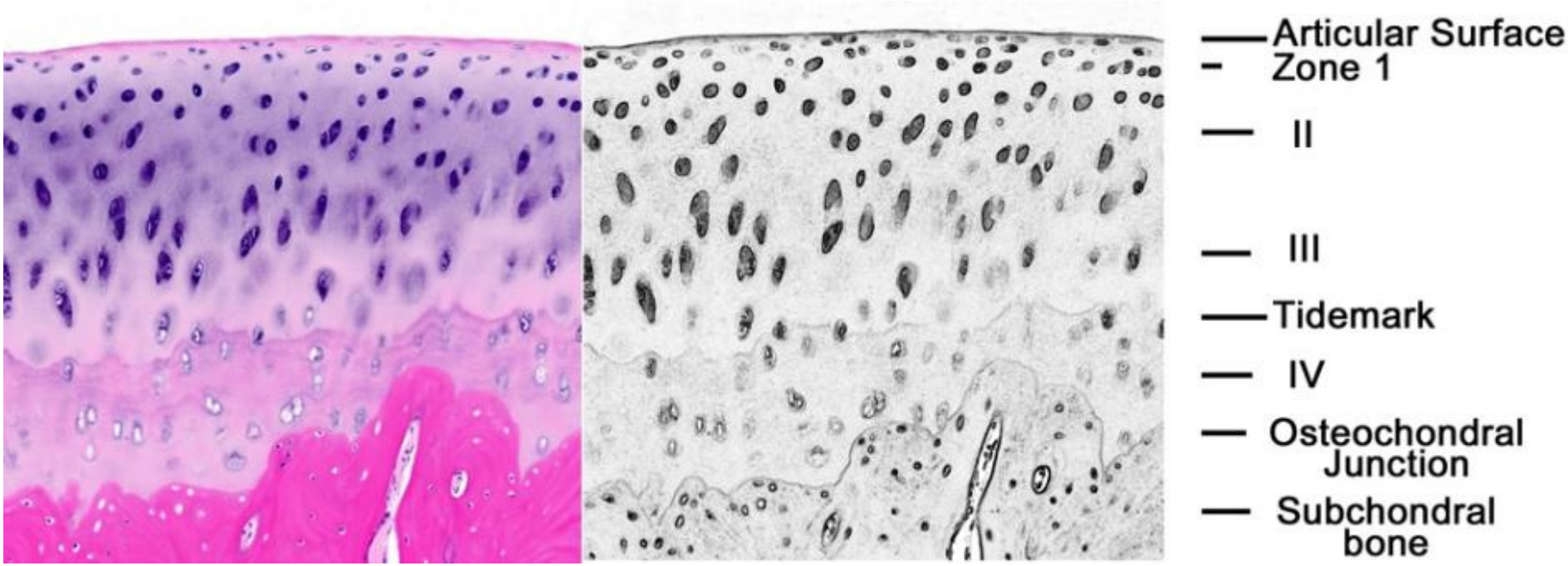

Figure 4. Articular cartilage, proximal femur, cynomolgus monkey. (a) H&E, coronal plane, decalcified section, (b) Black and white reproduction of image (a) demarcating zones I-IV and location of tidemark.

Reproduced from Haschek & Rousseaux Handbook of Toxicologic Pathology, 3rd Ed. Academic Press 2013.

Table 1. Selected Articular Cartilage (Stifle/Knee) Characteristics.

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Species	Average Cartilage Thickness, mm	Cartilage Anatomy
Mouse[a]	0.03	• Thick layer of calcified cartilage (as thick or thicker than noncalcified cartilage layer) • No distinct superficial, transitional, and radial zones of chondrocytes • Partial-thickness lesions not recognized
Rat[a]	0.1	• Radial zone makes up nearly two-thirds of cartilage thickness
Guinea pig[a]	0.3–0.4	• Superficial, transitional, radial, and deep zones of chondrocytes • Characteristic apoptosis of deep zone chondrocytes with naturally occurring disease
Rabbit[b]	0.2-0.7	• Greater chondrocyte density than that of other species • Thickness and cellularity of transitional and radial zones of chondrocytes highly variable even within a single joint
Dog[c]	0.6–1.3	• Transitional zone can be divided into an outer zone (thinner) and inner zone, with larger cell size and greater cell concentration in the outer zone
Sheep; goat[d]	0.4–0.5; 0.7–1.5	• Superficial, transitional, radial, and deep zones of chondrocytes • Similar anatomy between species, except for thickness of noncalcified cartilage
Horse[e]	1.5–2.0	• Cellular structure, biochemical makeup, and biomechanical properties most similar to human cartilage among animal species • Calcified cartilage layer easily identified, approximately 200 μm thick
Human	2.2–2.5	• Calcified cartilage layer <10% the thickness of noncalcified cartilage layer • Distinct superficial, transitional, radial, and deep zones of chondrocytes • Superficial and deep chondrocyte zones thinner than transitional and radial zones

[a]Critical defect size: unknown.
[b]Critical defect size: 3 mm (4–5 mm recommended).
[c]Critical defect size: 4 mm (≥5 mm recommended)
[d]Critical defect size: 6–7 mm (for both sheep and goat).
[e]Critical defect size: 9 mm.

A. M. McCoy Vet Pathol 2015;52:803-818

Study design considerations for translatability

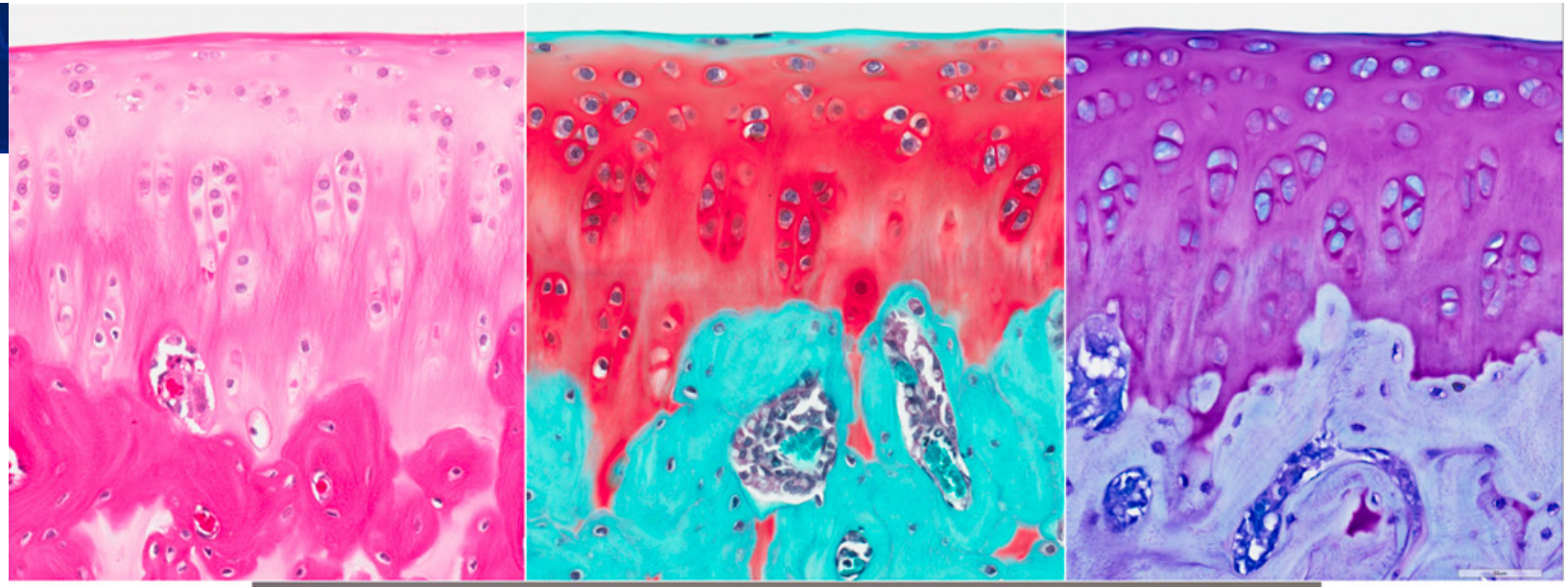
- Acute vs. Chronic changes
  - Structural responses
  - Pain responses
- Interventions
  - Prevention regimens
  - Treatment regimens
- Age of animals
  - Responses of young adult vs. older adult

In life assessments

- Pain Assessments
  - Electrophysiology
  - Von Frey hair algesiometry
    - Assesses referred pain
  - Activity
- Gait Analysis
  - Baseline data
- Imaging
  - Baseline imaging in large animals
- Biomarkers

Ex-vivo Assessments

- Histopathology
  - Semi-quantitative scoring (OARSI initiative)
  - Articular cartilage, subchondral bone, osteophytes, synovial membrane
  - Consistency of sections/staining is critical
    - Decalcified step sections
    - Small animals: Stifle joint in-toto
    - Large animals: Distal femur, proximal tibia
      - Crucial to examine grossly to identify region of interest
    - Preferred stains to assess cartilage integrity
      - Toluidine Blue
      - Safranin O



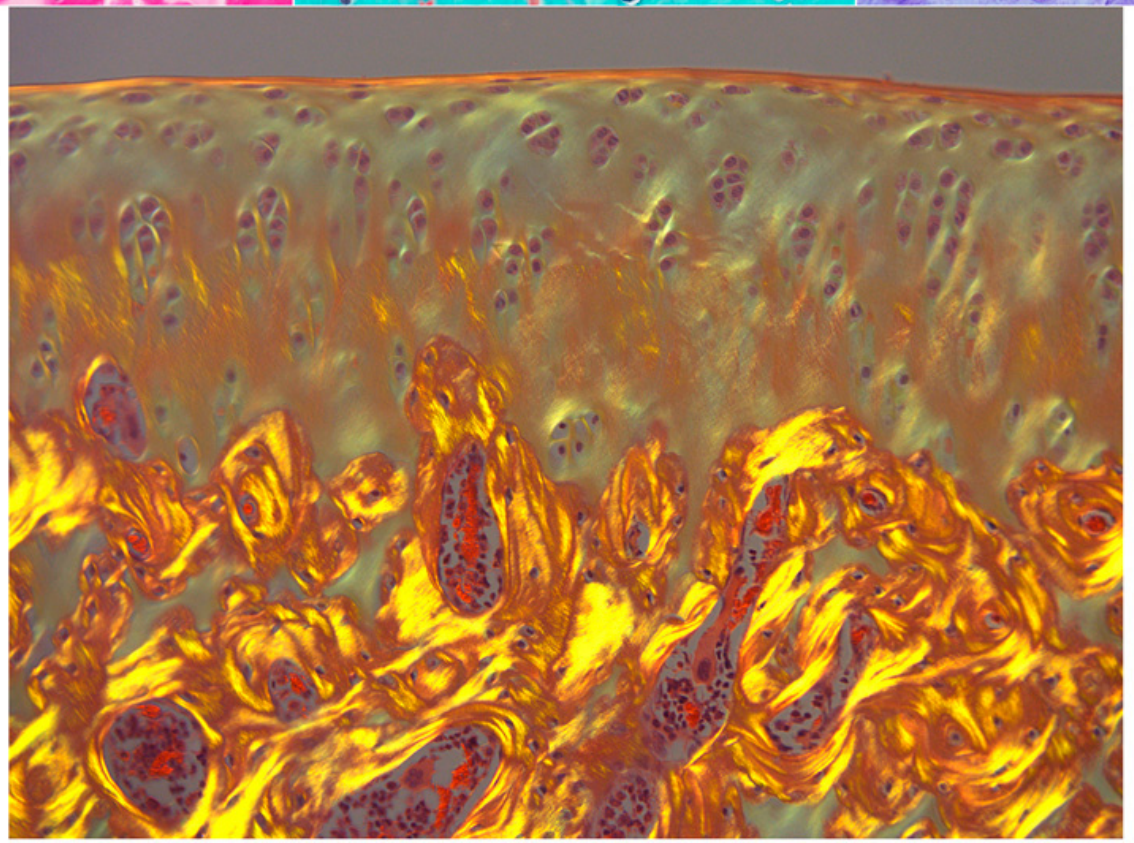


Fig 3. Rat articular cartilage stained with (e) H&E, (f) Safranin O (g) Toluidine blue.
(h) Polarized light image reveals collagen fiber orientation in subchondral lamellar bone.

Reproduced from Haschek & Rousseaux Handbook of Toxicologic Pathology, 3rd Ed. Academic Press 2013.

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Example of Safranin-O staining in a model of Mouse OA

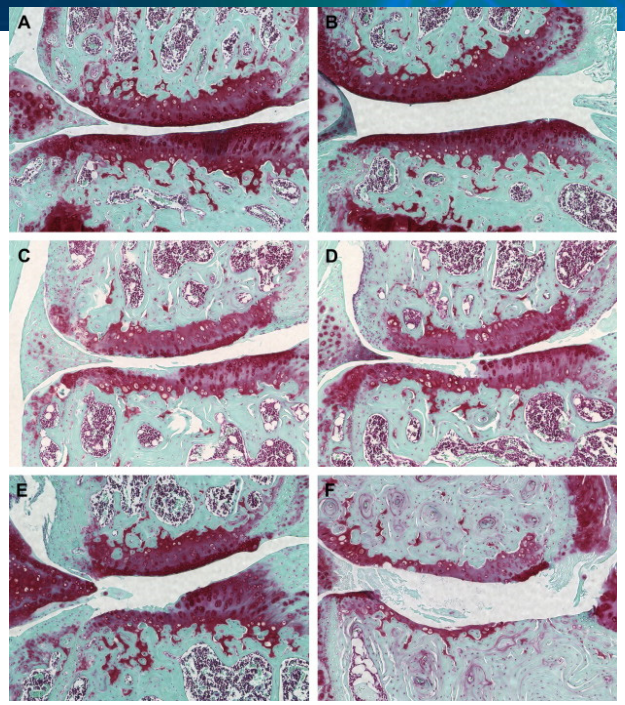


Fig. 1. Safranin-O photomicrographs showing the MFC (above) and MTP (below) and the medial meniscus (left), displaying a variety of OA severity and semi-quantitative scores. First score represents MFC, second score is MTP; (A) 0, 0.5; (B) 0, 1; (C) 0.5, 2; (D)...

S.S. Glasson, M.G. Chambers, W.B. Van Den Berg, C.B. Little

The OARSI histopathology initiative – recommendations for histological assessments of osteoarthritis in the mouse

Osteoarthritis and Cartilage, Volume 18, Supplement 3, 2010, S17–S23

http://dx.doi.org/10.1016/j.joca.2010.05.025

Example of proposed semi-quantitative scoring system in the mouse (OARSI)

Grade	Loss of cartilage proteoglycan
0	Normal staining of non-calcified cartilage
1	Decreased but not complete loss of toluidine blue staining over 1-100 % of the articular surface
2	Complete loss of toluidine blue staining in the non-calcified cartilage extending to <25% of the articular surface
3	Complete loss of toluidine blue staining in the non-calcified cartilage extending to 25-50% of the articular surface
4	Complete loss of toluidine blue staining in the non-calcified cartilage extending to 50-75% of the articular surface
5	Complete loss of toluidine blue staining in the non-calcified cartilage extending to >75% of the articular surface

S.S. Glasson, M.G. Chambers, W.B. Van Den Berg, C.B. Little; **The OARSI histopathology initiative – recommendations for histological assessments of osteoarthritis in the mouse.** Osteoarthritis and Cartilage, Volume 18, Supplement 3, 2010, S17–S23

Controls in OA Models

- Techniques which target one joint (usually the stifle joint) allow use of the contralateral limb as a self-control
  - However, if effects on the targeted joint are large, the contralateral limb may show compensatory changes
    - Inclusion of 'sham' and/or untreated control groups!
- Nearly all methods of assessment are semi-quantitative; blinding is highly recommended to minimize assessor bias.

Categories of animals models of OA

- Artificially induced
  - Surgical methods (traumatic OA)
  - Chemical methods (chondrocyte destruction)
- Spontaneous
  - Age related
  - Genetic predisposition
- Genetically modified

Surgically Induced Models of OA

- Creation of joint defects causing joint instability
  - Medial Menisectomy/Tear in Rodents
    - Reproducible
    - Weight bearing maintained
    - Relatively slow onset/progression of OA (weeks to months)
  - Other transection models
    - Collateral ligaments
    - Patellar tendons
    - Combinations
  - Surgical consistency is critical
  - 'Sham' procedures may vary from lab to lab.

Cartilage matrix changes in the rat following MMT

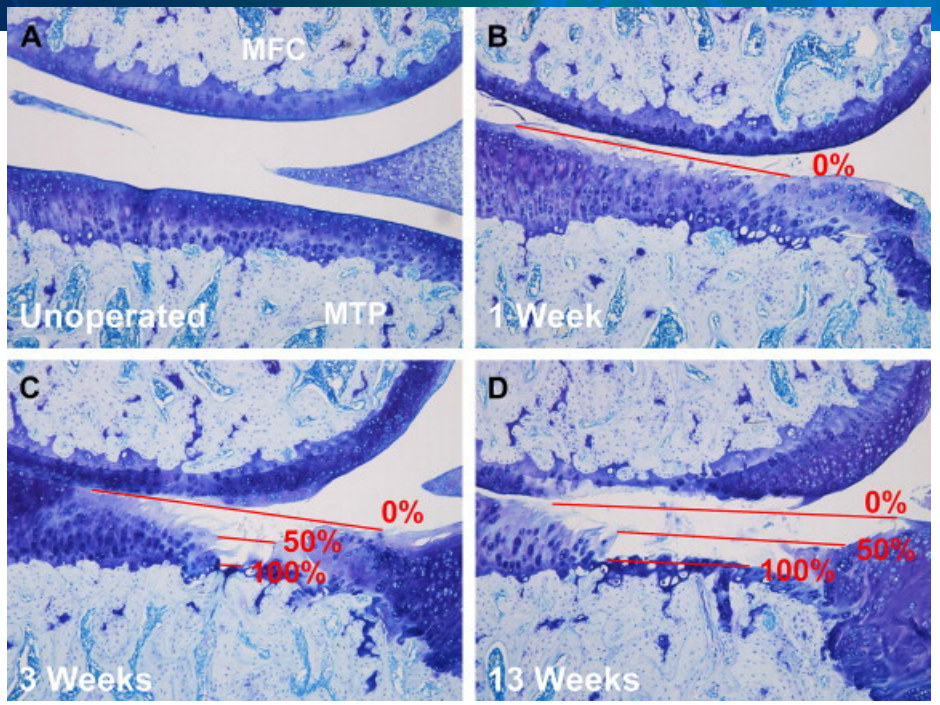


Fig. 3. #1 Cartilage matrix loss width. Histological sections of the MFC and the MTP of unoperated rats (A.) and rats with OA lesions at 1 (B.), 3 (C.) and 13 (D.) weeks following MMT. For evaluation, the widths of collagen matrix loss are measured in relation...

N. Gerwin, A.M. Bendele, S. Glasson, C.S. Carlson

The OARSI histopathology initiative – recommendations for histological assessments of osteoarthritis in the rat

Osteoarthritis and Cartilage, Volume 18, Supplement 3, 2010, S24–S34

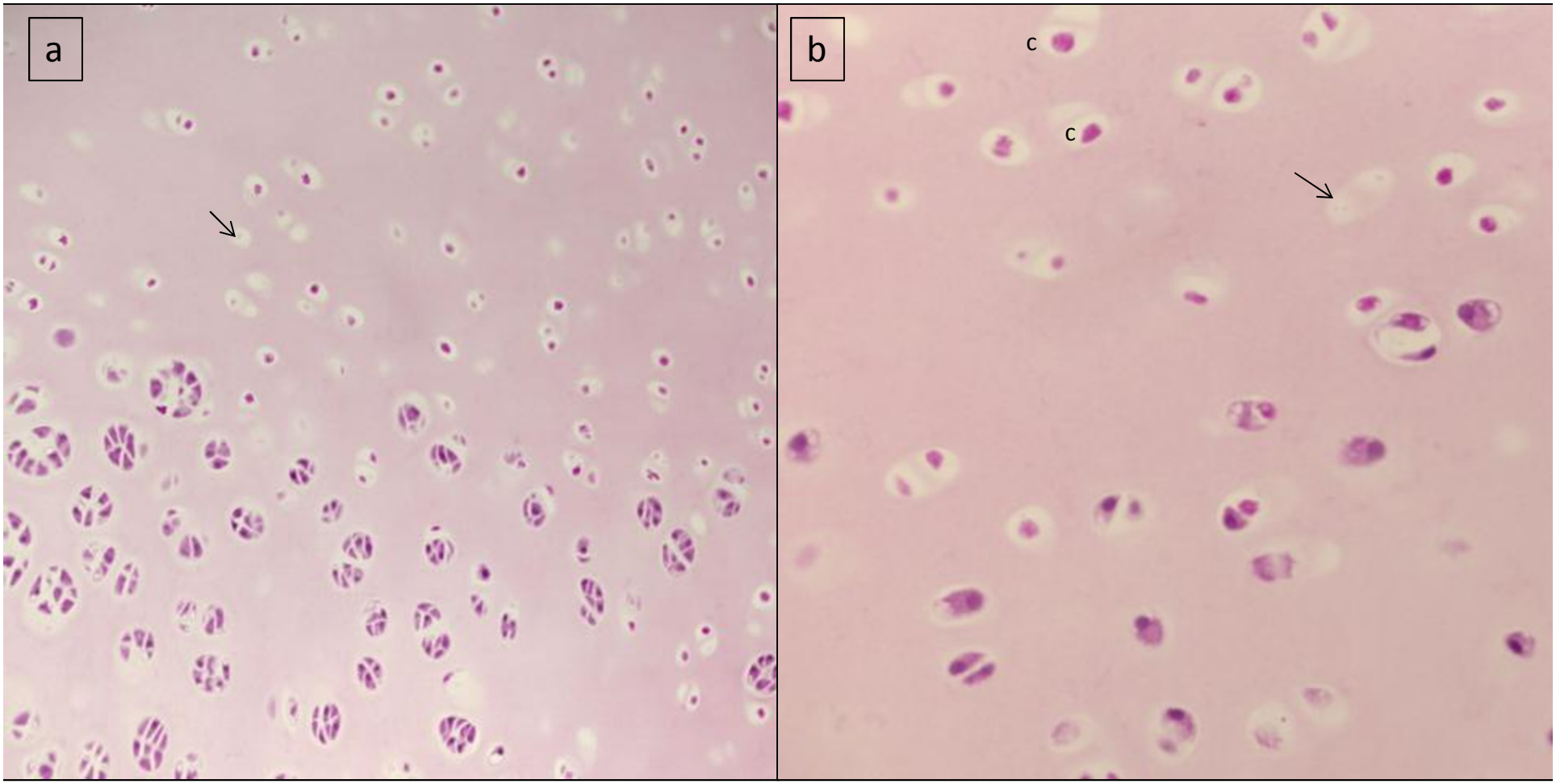
http://dx.doi.org/10.1016/j.joca.2010.05.030

Chemically Induced Models

- Direct chemical damage to articular chondrocytes
  - Iodoacetate Injection Model in Rodents
    - Location of maximum cartilage damage can be quite variable
    - Dose matters!
    - Rapid onset of severe OA (days to weeks)
    - Most useful as a model of pain in OA

Chondronecrosis in the pig

Figure 34 a, b. Ischemic chondronecrosis/osteochondrosis, medial femoral condyle of 12-week-old domestic pig. Chondrocyte necrosis is evident as empty chondrocyte lacunae (arrows), or lacunae with degenerate, eosinophilic chondocytes, lacking cellular detail (c). Adjacent areas (see a, left) show chondrocyte cloning.
Images courtesy of Dr. Cathy Carlson, University of Minnesota, St Paul, MN. Reproduced from Haschek & Rousseaux Handbook of Toxicologic Pathology, 3rd Ed. Academic Press 2013.



Spontaneous Animal Models of OA

- Mouse
  - STR/ort, C57Bl/6, BALB/c
- Guinea Pig
  - Dunkin-Hartley
- Non-human primates
  - Cynomolgus Monkey
    - Cartilage changes by 9 years of age
- Dog
  - Hip dysplasia, elbow dysplasia, ACL
- Horse
  - Carpal joint

Spontaneous OA in the Guinea Pig

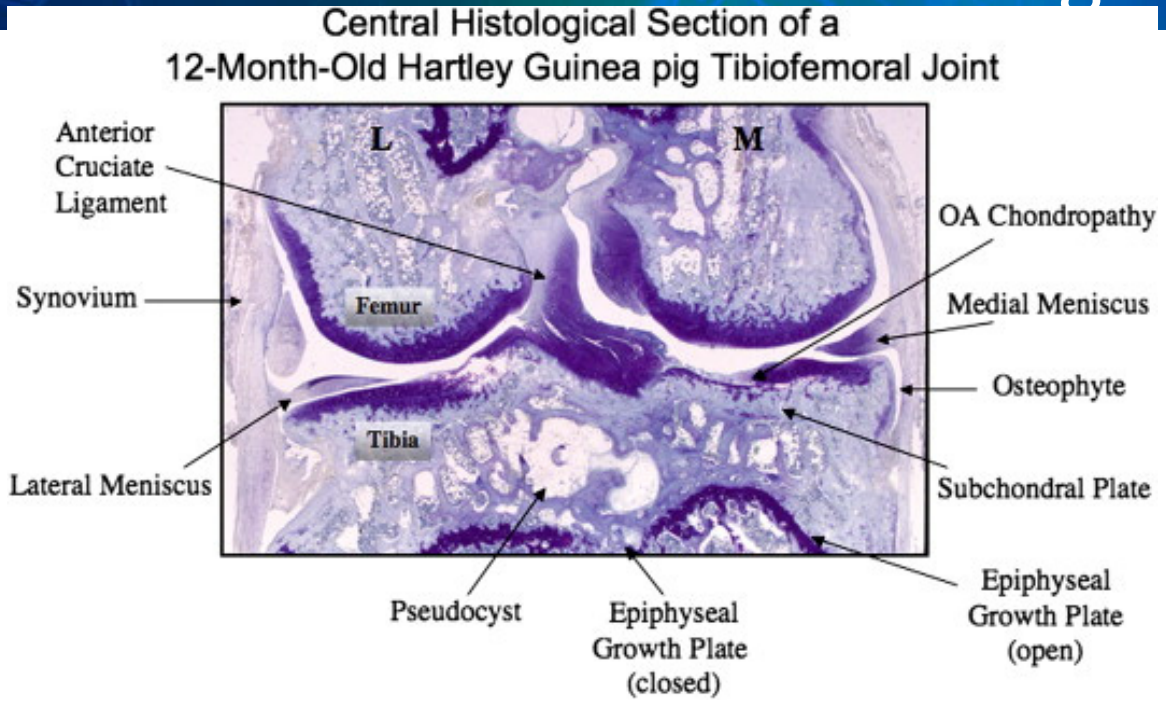


Fig. 1. Guinea pig knee joint structures. A frontal section through a 12-month-old guinea pig knee joint to demonstrate its structures and the landmarks and features typical of advanced OA at the center of the tibiofemoral joint (M = medial; L = lateral).

V.B. Kraus, J.L. Huebner, J. DeGroot, A. Bendele

The OARSI histopathology initiative – recommendations for histological assessments of osteoarthritis in the guinea pig 1

Osteoarthritis and Cartilage, Volume 18, Supplement 3, 2010, S35–S52

http://dx.doi.org/10.1016/j.joca.2010.04.015

Spontaneous OA in a monkey

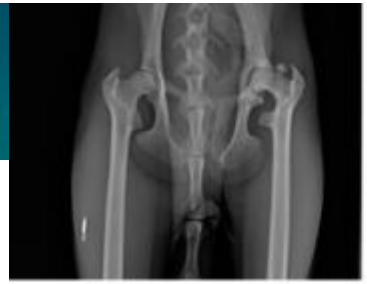


Figure Chondrolysis of hip joint in young Cynomolgous monkeys. a) radiograph showing irregular surface of right femoral head compared to normal left side. b) rough dull cartilage of right femoral head compared to normal left side.
c) e)g) Unaffected left acetabulum
d) f) h) Affected right acetabulum has degenerating, fibrillated cartilage with loss of red Safranin O staining

Reproduced from Haschek & Rousseaux Handbook of Toxicologic Pathology, 3rd Ed. Academic Press 2013.

Comparison of spontaneous OA in the dog vs. human

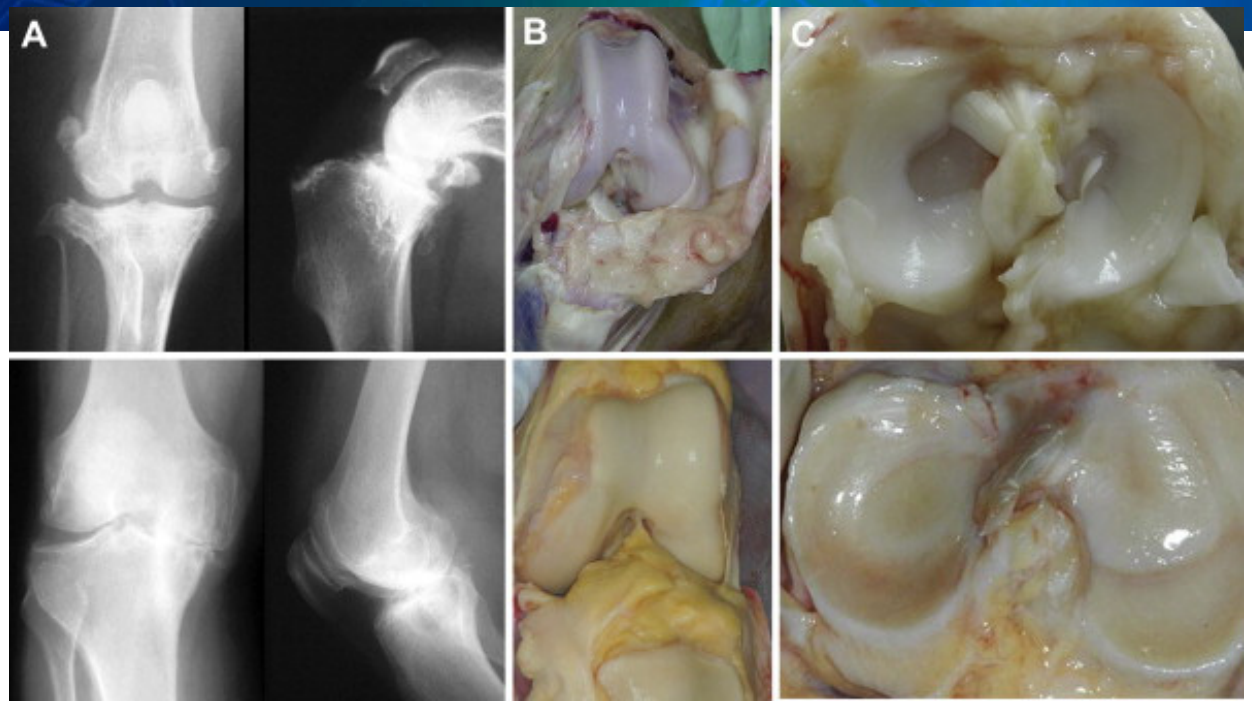


Fig. 1. Comparative radiographic (A) and gross (B, C) anatomy of the canine stifle (top row) and human knee (bottom row). The radiographic images show osteoarthritic joints while the gross images show normal patellofemoral joints (B) and normal tibial plateaus...

J.L. Cook, K. Kuroki, D. Visco, J.-P. Pelletier, L. Schulz, F.P.J.G. Lafeber

The OARSI histopathology initiative – recommendations for histological assessments of osteoarthritis in the dog

Osteoarthritis and Cartilage, Volume 18, Supplement 3, 2010, S66–S79

http://dx.doi.org/10.1016/j.joca.2010.04.017

Summary

- Presentation of osteoarthritis in human patients is usually at an advanced stage regardless of cause
- Animal models of OA whether induced or spontaneous are used to study OA at an early stage
- No animal model perfectly simulates OA in humans
- Choose the most appropriate animal model for your investigation
- Consider carefully all variables (procedure, controls, assessments) to create the most robust study design.

Recent Review Articles

- Kuyino EL, Narayanan G, Nair LS, et al. Animal models of osteoarthritis: classification, update, and measurement of outcomes. (2016) J Orthopaed Surg Res 11: 19, 27 pages
- McCoy AM. Animal models of osteoarthritis: comparisons and key considerations. (2015) Vet Pathol 52:803-818.
- Thysen S, Luyten FP, Lories RJU. Targets, models and challenges in osteoarthritis research. (2015) Disease Models & Mechanisms 8:17-30.
- Lampropoulou-Adamidou K, Lelovas P, Karadimas EV, et al. Useful animal models for the research of osteoarthritis (2014) Eur J Orthop Surg Traumatol, 24: 263-271
- Fang H and Beier F. Mouse models of osteoarthritis: modelling risk factors and assessing outcomes. (2014) Nat Rev Rheumatol 10: 413-421
- Malfait A-M, Little CB, McDougall JJ. A commentary on modelling osteoarthritis pain in small animals. (2013) Osteoarthritis Cartilage 21(9): 1316-1326.
- Teeple E, Jay GD, Elsaid KA, et al. Animal models of osteoarthritis: challenges of model selection and analysis. (2013) AAPS J 15: 438-446
- Gregory MH, Capito N, Kuroki K, et al. A review of translational animal models for knee osteoarthritis. (2012) Arthritis; Article ID 764621, 14 pages.

Additional References

- OARSI Histopathology Initiative published in 2010 as a special issue of Osteoarthritis & Cartilage (volume 18, Supplement 3).
  - Open Access

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