Development of an in vitro model of injury-induced osteoarthritis in cartilage explants from adult horses through application of single-impact compressive overload

Christina M. Lee, PhD; John D. Kisiday, PhD; C. Wayne McIlwraith, BVSc, PhD, DSc; Alan J. Grodzinsky, ScD; David D. Frisbie, DVM, PhD

Objective—To develop an in vitro model of cartilage injury in full-thickness equine cartilage specimens that can be used to simulate in vivo disease and evaluate treatment efficacy.

Sample—15 full-thickness cartilage explants from the trochlear ridges of the distal aspect of the femur from each of 6 adult horses that had died from reasons unrelated to the musculoskeletal system.

Procedures—To simulate injury, cartilage explants were subjected to single-impact uniaxial compression to 50%, 60%, 70%, or 80% strain at a rate of 100% strain/s. Other explants were left uninjured (control specimens). All specimens underwent a culture process for 28 days and were subsequently evaluated histologically for characteristics of injury and early stages of osteoarthritis, including articular surface damage, chondrocyte cell death, focal cell loss, chondrocyte cluster formation, and loss of the extracellular matrix molecules aggrecan and types I and II collagen.

Results—Compression to all degrees of strain induced some amount of pathological change typical of clinical osteoarthritis in horses; however, only compression to 60% strain induced significant changes morphologically and biochemically in the extracellular matrix.

Conclusions and Clinical Relevance—The threshold strain necessary to model injury in full-thickness cartilage specimens from the trochlear ridges of the distal femur of adult horses was 60% strain at a rate of 100% strain/s. This in vitro model should facilitate study of pathophysiologic changes and therapeutic interventions for osteoarthritis. (Am J Vet Res 2013;74:40–47)

In adults, cartilage has limited potential for natural repair and thus injuries typically result in the progression of osteoarthritis. The study of osteoarthritis is of particular interest in equine medicine because joint injury and joint disease are major causes of lameness and consequent wastage of athletic horses. Histologic lesions associated with osteoarthritis can include fissure formation in the articular cartilage surface, chondrocyte cell death, focal cell loss, chondrocyte cluster formation, and loss of ECM such as proteoglycans and collagen fibrils.

In vitro models of cartilage injury can enable investigation of the responses of cartilage to traumatic injury in a highly controlled and reproducible manner. Additionally, well-developed in vitro models allow for high throughput and inexpensive preliminary testing of approaches to treat and prevent the progression of osteoarthritis.

Many in vitro models of cartilage injury exist and have been summarized in literature reviews. Investigators have used adult and immature cartilage explants from various animal species, including humans. Immature cartilage tissue subjected to injurious compression has been used to gain information regarding traumatic injury in immature human knees. Studies performed with adult articular cartilage subjected to injurious loads in vitro have relevance to early, middle, and later stages of osteoarthritis. These changes in cartilage in vitro have been evaluated and compared with results

1. In adults, cartilage has limited potential for natural repair and thus injuries typically result in the progression of osteoarthritis.

2. The study of osteoarthritis is of particular interest in equine medicine because joint injury and joint disease are major causes of lameness and consequent wastage of athletic horses.

3. Histologic lesions associated with osteoarthritis can include fissure formation in the articular cartilage surface, chondrocyte cell death, focal cell loss, chondrocyte cluster formation, and loss of ECM such as proteoglycans and collagen fibrils.

4. In vitro models of cartilage injury can enable investigation of the responses of cartilage to traumatic injury in a highly controlled and reproducible manner.

5. Additionally, well-developed in vitro models allow for high throughput and inexpensive preliminary testing of approaches to treat and prevent the progression of osteoarthritis.

6. Many in vitro models of cartilage injury exist and have been summarized in literature reviews.

7. Investigators have used adult and immature cartilage explants from various animal species, including humans. Immature cartilage tissue subjected to injurious compression has been used to gain information regarding traumatic injury in immature human knees.

8. Studies performed with adult articular cartilage subjected to injurious loads in vitro have relevance to early, middle, and later stages of osteoarthritis. These changes in cartilage in vitro have been evaluated and compared with results.

Received June 29, 2011.
Accepted May 29, 2012.
From the Orthopaedic Research Center, Department of Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO 80523 (Lee, Kisiday, McIlwraith, Frisbie), and the Center for Biomedical Engineering, Department of Biological Engineering, School of Engineering, Massachusetts Institute of Technology, Cambridge, MA 02139 (Grodzinsky).
Supported in part by the Colorado State University College Research Council.
Address correspondence to Dr. Frisbie (Davidfrisbie@colostate.edu).
of animal studies in which impact loads have been directly applied to joint cartilages in vivo.

The purpose of the study reported here was to develop an in vitro model of cartilage injury in full-thickness cartilage specimens (containing the superficial, middle, and deep cartilage zones) extracted from the stifle joint of adult horses by use of a computer-controlled single-impact model of unconfined compression. Our objective was to determine the degree of strain sufficient to reproducibly induce pathological change in cartilage that would mimic early osteoarthritic changes in vivo.

Materials and Methods

Specimens—Cartilage specimens were extracted from the stifle joints of 6 equine cadavers within 16 hours after death. The horses had been between 3 and 10 years of age and had died for reasons unrelated to the musculoskeletal system and unrelated to this study.

A mosaicplasty osteochondral grafting system was used to extract at least 15 osteochondral plugs (diameter, 4.5 mm) that included the intact superficial cartilage zone from the proximal region of the trochlear ridges of the left and right femurs from each horse. Specimens from each horse were pooled. Cartilage was removed from the subchondral bone at the calcified and noncalcified cartilage junction immediately after extraction from the joint, followed by incubation in growth medium (Dulbecco modified Eagle medium, 10% fetal bovine serum [vol/vol], ascorbic acid [20 µg/mL], 1% penicillin and streptomycin [vol/vol], 10mM HEPES, 0.1mM nonessential amino acids, 0.4mM proline, and amphotericin B [0.25 µg/mL]) for 48 hours prior to injury.

Mechanical injury induction—Just prior to injury induction, the thickness of each cartilage plug (plane perpendicular to the articular surface) was measured to 0.01-mm accuracy with digital calipers. For each horse, 3 cartilage specimens were assigned to each of 5 groups: 1 free-swell control and 4 injury groups at various degrees of strain.

Cartilage plugs assigned to injury groups were removed from medium and placed into a sterile polysulphone loading chamber consisting of a well aligned coaxially with an impermeable platen (diameter, 10 mm) in the absence of medium (Figure 1). A test system was used to apply an initial compressive tare load of 2 N. After creep equilibrium was attained, an injurious compression at a rate of 100% strain/s was applied until 50%, 60%, 70%, or 80% final cartilage strain was achieved (Figure 2). Compression was then released at the same rate, and plugs were immediately placed in culture medium for 28 days. All medium was changed at similar intervals (every 2 to 3 days). Control plugs were kept in medium throughout the study, which was changed at a similar rate.

Twenty-eight days after cartilage injury, all cartilage plugs, including those in the control group, were examined grossly and microscopically and digital photographic images were obtained to document the integrity of the articular surface and presence of fissures. Specimens were sectioned across the diameter perpendicular to the fissures when fissures were evident or arbitrarily across when not. Half of each specimen was placed into neutral-buffered 10% formalin for fixation. The remaining half was embedded in cryoembedding medium, snap-frozen in liquid nitrogen, and stored at –80°C for further use.

Histologic evaluation—Formalin-fixed specimens were processed, paraffin embedded, and sectioned (thickness, 5 µm) with a microtome, then transferred to slides. One slide (containing 2 tissue sections) was evaluated for each stain used. Four sections each from an injured and control specimen were stained with H&E to evaluate cellular changes or SOFG to identify changes in GAG content. Digital images of SOFG-stained sections and image management software were used to measure fissure size, defined as the area within the fissure, and fissure depth, determined as the depth of the fissure as a percentage of the total height of each specimen. The software was calibrated to the microscope and camera.

IHC evaluation—Immunohistochemical staining was performed to detect aggrecan and collagen (type I

Figure 1—Photograph of a polysulphone loading chamber used to evaluate the effects of injurious compression on cartilage specimens from the trochlear ridge of the femur from an adult horse. For injury induction, a cartilage specimen was placed into the well of the chamber directly under the platen.

Figure 2—Representative stress-strain curve showing the results of a testing machine used to load and induce compressive injury in the cartilage specimens described in Figure 1.
to assess repair and type II to assess unaffected matrix). Briefly, frozen embedded specimens were sectioned
(thickness, 8 µm) with a cryostat and mounted onto
adhesive slides. Mouse antibodies raised against
aggrecan at a 1:20 dilution or collagen types I and II by
use of undiluted supernatant were applied, followed by
goat anti-mouse secondary antibody conjugated with
horseradish peroxidase at a 1:500 dilution. For nega-
tive control specimens, additional sections were probed
with mouse serum at a concentration equal to that of
the primary antibody.

Scoring of cartilage sections—All histologic and
IHC sections were scored with the aid of a grading scale
to determine the severity of osteoarthritic charac-
teristics in equine cartilage, with minor modifications.
Necrotic nuclei were not present in the cartilage sec-
tions; instead, there were empty lacunae attributed to
chondroptosis, which is a process of chondrocyte cell
death that results in the presence of an empty lacuna. Therefore, as opposed to evaluating chondrocyte necro-
sis, chondrocyte cell death was evaluated and defined
by the presence of lacunae lacking discernible nuclei.
Focal cell loss was measured in a manner similar to
the established grading scale but defined as the pres-
ence of distinct regions devoid of discernible nuclei,
and chondrocyte cluster formation (lacunae containing
chondrocytes with > 1 nucleus) was evaluated as de-

No specimen was separately graded for each
osteochondrosis characteristic in the superficial, middle,
and deep regions as well as at the region adjacent to
the fissure (defined as the region 3 times the area of
the largest fissure).

In brief, each region was assigned a grade for each
osteochondrosis characteristic that corresponded to the
severity by which the specimen deviated in histologic
appearance from undamaged articular cartilage as fol-
lows: 0 = no change or abnormality, 1 = slight devi-
ance, 2 = mild deviance, and 3 = moderate deviance
(the grade for severe deviance [4] was not used). For
each osteochondrosis characteristic, scores for each region
(superficial, middle, deep, and fissures) were evaluated
separately or, to gain an understanding of the section
as a whole, were summed together for each slide to
provide a cumulative score with a maximum score of
12. All slides were graded by the consensus of 2 evalu-
ators (CML and DDF), who were unaware of group
assignment.

Histologic and IHC-stained sections were used to
evaluate changes in the ECM. The SOFG method is an
indirect method for identifying the presence of GAG
because GAG chains in healthy articular cartilage are
negatively charged and the SOFG stain is cationic. A
decrease in the degree of SOFG staining was interpreted
as indicating a decrease in GAG content, and thus the
SOFG-stained sections were used to identify any regions
with less GAG content than in control specimens. To
identify ECM molecules more specifically, IHC-stained
sections were used to assess any changes in aggregan,
collagen type II, and collagen type I content, compared
with the content in control sections.

Statistical analysis—Statistical analysis was con-
ducted to determine differences in IHC and histologic
staining results accounting for both injury and region,
with horse as a random variable in a generalized linear
mixed model. This method of analysis allowed for intra-
group comparisons as well as the ability to compare
static culture with the various injury groups to account
for any effects static culture may have had. Predictive
F values were used to identify statistical differences
among injury and control groups or among cartilage
regions, and specific comparisons (when indicated by a
significant F test result) were made with a least squares
means procedure. Values of P < 0.05 were considered
significant for all analyses.

Results

Pathological changes in the cartilage matrix—The
mean ± SD values of characteristics of full-thickness cartilage
specimens obtained from the trochlear ridge of femurs from 6 adult
horses was summarized (Table 1). Peak stress was significantly
(P < 0.001) affected by strain, ranging from 11.39 ± 5.55 MPa for cartilages specimens sub-
jected to 50% strain to 22.29 ± 4.74 MPa for specimens subjected to 80% strain. Compression at 60% and 70% strain induced similar stresses of 14.68 ± 5.55 MPa and 16.02 ± 5.52 MPa, respectively. All specimens devel-
oped fissures when compressed to 70% strain. Most of the specimens developed fissures when compressed to 60% and 80% strain (14/18 and 7/9, respectively), and
9 of 18 developed fissures when compressed to 50% strain.

Fissure size and depth were significantly smaller
in specimens compressed to 50% than in those com-
pressed to 70% strain (P = 0.026 and P = 0.030, re-
spectively) and 80% strain (P = 0.002 and P = 0.014, re-
spectively). All specimens compressed to 50% and
60% strain remained acceptable for use in the study; how-
ever, some specimens in the 70% and 80% strain
groups were lost during compression because the maxi-

| Table 1—Mean ± SD values of characteristics of full-thickness cartilage specimens from the trochlear ridge of femurs from 6 adult horses that were subjected to various degrees of strain. |
|---------------------------------|----------------|----------------|----------------|----------------|-----------------|
| Variable                        | 50% (n = 18)   | 60% (n = 18)   | 70% (n = 13)   | 80% (n = 9)    | P value         |
| Specimen height (mm)            | 1.65 ± 0.55a   | 1.73 ± 0.55a   | 1.73 ± 0.50a   | 1.72 ± 0.45a   | 0.89            |
| Stress achieved (MPa)           | 11.39 ± 5.55a  | 14.88 ± 5.55a  | 16.02 ± 5.32a  | 22.29 ± 4.74a  | < 0.001         |
| Fissure rate (%)                | 50%            | 78%            | 100%           | 78%            | < 0.001         |
| Fissure size (µm³)              | 309.11 ± 1,036.51a | 619.14 ± 756.96a | 1,031.16 ± 890.37a | 1,595.40 ± 1,031.16a | < 0.001         |
| Fissure depth (µm)              | 16.07 ± 14.12a | 23.93 ± 10.01a | 25.54 ± 9.07a  | 29.19 ± 11.55a | 0.07            |

a Values with different superscript letters differ significantly (P < 0.05) from each other.
mum load reached during compression exceeded the capacity of the 500-N load cell.

**Strain and pathological change**—Compared with control specimens, GAG content was significantly (all \( P < 0.001 \)) lower in specimens compressed to 60%, 70%, and 80% and type II collagen content was significantly (\( P = 0.003 \)) lower in specimens compressed to 60% strain (Table 2). No effect of compression on type I collagen or aggrecan content was induced a significant increase in chondrocyte cell death, focal cell loss, and chondrocyte cluster formation, compared with values in control specimens and specimens compressed to 50% strain. When scores for all cellular changes were cumulatively evaluated, all compressed specimens had more severe total cellular pathological scores than did control specimens, with severity increasing with increasing degree of strain. In summary, specimens compressed to \( \geq 60\% \) strain had the greatest amount of macroscopic and microscopic cellular pathological change, compared with control specimens (Figure 3).

**Regional pathological change**—Chondrocyte cluster formation was significantly affected by region and injury (Table 3). For all injury groups, there was a significant increase in cluster formation in the deep region, compared with in all other regions. Injury induced through 60% strain was the only injury group with a significant difference in all 3 regions of cartilage. Independent of injury, severity of changes characteristic of osteoarthritis differed significantly by region when all specimens (control and 50%, 60%, 70%, and 80% strain) were analyzed as 1 group for evaluation of regional (superficial, middle, and deep cartilage) differences. Glycosaminoglycan and aggrecan content were most severely decreased in the deep cartilage region, compared with in all other regions. In all comparisons, the fissure region had the same severity of osteoarthritis characteristics as did the superficial and deep regions than in the middle region.

In addition, specimens with fissures were analyzed separately to determine whether the severity of osteoarthritis characteristics in the region around the fissure differed significantly from the severity in other regions. In all comparisons, the fissure region had the same severity of osteoarthritis characteristics as did the superficial and deep regions, except for chondrocyte cluster formation, which was more severe in the middle region than in the superficial zone (Figure 5).

### Table 2—Mean ± SD cumulative lesion severity scores for superficial, middle, and deep cartilage layers and fissures in full-thickness cartilage specimens from the horses in Table 1 that were or were not (control) subjected to mechanical compression at various degrees of strain.

<table>
<thead>
<tr>
<th>Injuy type</th>
<th>Control (n = 18)</th>
<th>50% (n = 18)</th>
<th>60% (n = 18)</th>
<th>70% (n = 13)</th>
<th>80% (n = 9)</th>
<th>( P ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>GAG</td>
<td>8.5 ± 1.95*</td>
<td>9.3 ± 1.95*</td>
<td>10.9 ± 1.91*</td>
<td>11 ± 1.77*</td>
<td>11 ± 1.47*</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Collagen type II</td>
<td>1.0 ± 4.03*</td>
<td>2.9 ± 5.19*</td>
<td>4.6 ± 5.32*</td>
<td>3.3 ± 4.74*</td>
<td>2.7 ± 3.94*</td>
<td>0.05</td>
</tr>
<tr>
<td>Collagen type I</td>
<td>0 ± 0.59*</td>
<td>0 ± 0.59*</td>
<td>0.2 ± 0.54*</td>
<td>0.2 ± 0.54*</td>
<td>0.4 ± 0.54*</td>
<td>0.24</td>
</tr>
<tr>
<td>Aggrecan</td>
<td>3.3 ± 3.9*</td>
<td>3.6 ± 3.7*</td>
<td>5.2 ± 3.69*</td>
<td>4.8 ± 3.43*</td>
<td>4.8 ± 3.48*</td>
<td>0.53</td>
</tr>
<tr>
<td>Cell death</td>
<td>3.3 ± 2.42*</td>
<td>4.7 ± 2.42*</td>
<td>7.3 ± 2.37*</td>
<td>7.8 ± 2.73*</td>
<td>8.5 ± 2.43*</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Focal cell loss</td>
<td>0.79 ± 2.46*</td>
<td>1.9 ± 2.46*</td>
<td>4.4 ± 2.42*</td>
<td>5.6 ± 2.57*</td>
<td>6.8 ± 2.43*</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Cluster formation</td>
<td>0.6 ± 2.20*</td>
<td>8.6 ± 2.20*</td>
<td>3.9 ± 2.12*</td>
<td>4.4 ± 2.06*</td>
<td>2.8 ± 2.22*</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Total severity</td>
<td>4.7 ± 5.51*</td>
<td>8.6 ± 5.51*</td>
<td>15.6 ± 5.51*</td>
<td>17.8 ± 5.05*</td>
<td>20 ± 5.4*</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

A score of 0 corresponds to unaffected, healthy cartilage, whereas a score of 12 corresponds to moderate severity of pathological change. \( a \)–\( d \)Within a row, values with different superscript letters differ significantly (\( P < 0.05 \)) from each other.
Injury to adult articular cartilage commonly leads to the degeneration of articular cartilage and the progression of osteoarthritis. Indeed, mechanical injury can induce matrix damage,11,13,21–25 chondrocyte death,13,15,16,23–33 and chondrocyte cluster formation,34 all of which are hallmark characteristics of osteoarthritis.

The goal of the present study was to induce and detect histologic changes in equine cartilage that emulate the natural changes found in osteoarthritis, with interest in determining regional histologic characteristics. Full-thickness cartilage explants harvested from the trochlear ridges of the distal aspect of the femur of adult horses were injured by an unconfined compression to 50%, 60%, 70%, or 80% strain and evaluated after the explants had been incubated for 28 days in culture medium for histologic changes characteristic of early osteoarthritis. The data acquired demonstrated that histologic change can be produced that is consistent with previous cartilage injury models typical of early osteoarthritis in vivo.4 For adult full-thickness cartilage from the trochlear ridge, it was necessary to compress explants by at least 60% strain, resulting in peak stresses $\geq 14.68 \pm 5.56$ MPa, to induce significant pathological changes, which included GAG loss, chondrocyte cell death, focal cell loss, and chondrocyte cluster formation.

The mechanical properties of cartilage are largely determined by the composition of the collagen-proteoglycan matrix,35,36 which in mature articular cartilage differs by cartilage region. The superficial zone has a relatively low proteoglycan content, and the collagen fibers are oriented parallel to the articular surface.37–39 The middle zone has the highest proteoglycan content and collagen fibers that are randomly oriented.40–42 The deep zone is also rich in proteoglycan content, but the collagen fibers are oriented perpendicular to the tidemark and subchondral bone.41,43

During unconfined compression, the collagen network is mainly responsible for controlling instantaneous deformation, whereas proteoglycans contribute to stiffness through regulation of osmotic pressure.44 As one would expect given the differential architecture, compressive load induces region-specific bending or crimping patterns of collagen fibers,45–47 which are associated with region-specific chondrocyte deformation.46 Therefore, we examined each region of cartilage independently and cumulatively to determine the effects of each degree of strain on ECM and cellular pathological change.

### Table 3—Mean ± SD severity scores for chondrocyte cluster formation by injury or strain in the cartilage specimens described in Table 2.

<table>
<thead>
<tr>
<th>Collagen region</th>
<th>Control (n = 18)</th>
<th>50% (n = 18)</th>
<th>60% (n = 18)</th>
<th>70% (n = 13)</th>
<th>80% (n = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Superficial</td>
<td>0.2 ± 0.76a</td>
<td>0.5 ± 0.76b,c</td>
<td>0.8 ± 0.76b,c</td>
<td>0.4 ± 0.72b,c</td>
<td>0.1 ± 3.26b,c</td>
</tr>
<tr>
<td>Middle</td>
<td>0.1 ± 0.76a</td>
<td>0.4 ± 0.76b,c</td>
<td>0.8 ± 0.76b,c</td>
<td>1.0 ± 0.72b</td>
<td>0.9 ± 3.26b,c</td>
</tr>
<tr>
<td>Deep</td>
<td>0.3 ± 0.76b,c</td>
<td>0.9 ± 0.76b</td>
<td>1.5 ± 0.76b,c</td>
<td>2.0 ± 0.72b</td>
<td>1.0 ± 3.26b,c</td>
</tr>
</tbody>
</table>

A score of 0 corresponds to unaffected, healthy cartilage, whereas a score of 3 corresponds to moderate cluster formation.

a–eWithin a row, individual injury scores with different superscript letters differ significantly ($P < 0.05$) from each other.
Chondrocyte cluster formation was the only histologic characteristic affected by compression magnitude and region. However, when pathological change was evaluated independent of compression magnitude, significant regional differences in severity became evident. Similar to what others have found, for most variables, the pathological change was most severe in the superficial region, compared with in other regions. This finding was not surprising given that the superficial region is more compliant under rapid loading, with a lower compressive modulus and greater permeability than other regions of the tissue. Indeed, when load is applied to cartilage of intact joints, most of the collagen fiber deformation occurs in the superficial zone, and with in vivo investigations, pathological change is also more concentrated in the region closest to the impact.

Cartilage injury and posttraumatic osteoarthritis result in a loss of proteoglycans and type II collagen content among injury groups, compression at ≥60% strain caused a significant decrease, compared with control specimens. In a previous study,14 collagen content was maintained longer than 28 days, we may have observed a significant regional change in type II collagen content. However, when pathological change was most severe in the superficial region, and in the middle zone of specimens compressed to ≥60% strain. However, only specimens compressed to 60% strain had significant increases in cluster formation in the superficial zone, compared with formation in control specimens. It is possible that compression to 70% or 80% strain generated localized stresses in the superficial region that prevented chondrocyte cluster formation or it could simply be that these high strains caused cell death, thereby preventing cluster formation. Interestingly, significant increases in chondrocyte cluster formation were observed in injured cartilage, even when there was little or no change in ECM composition. That finding suggested that although chondrocyte clusters are typically found in diseased or damaged cartilage at a time when there is a loss of ECM macromolecules, cluster formation may form in response to a factor that is independent of ECM degradation. The ability to reproducibly promote cluster formation with the in vitro model used in our study will allow for highly controlled investigations into the role these clusters play in the progression of osteoarthritis.

The objective of the present study was to develop an in vitro model of cartilage injury that made use of full-thickness equine cartilage and computer-controlled uniaxial unconfined compression. Certain limitations to this type of work should be taken into consideration but are difficult to avoid. First, the data were generated from fresh cadaveric tissue sources and, consequently, biological variation could be expected in the material properties of the cartilage and the response of the cartilage to injury between horses and within the joint of each horse. To examine interanimal variation, we included tissue from 6 horses and used a mixed-model approach to interpret the results to control for horse as a random variable.

To minimize the effects of variability in cartilage material properties by location within each horse, specimen collection was limited to a proximal region of the trochlear ridges that represents approximately 15% of the articular cartilage surface area of the joint. This small region was specifically chosen because the entire region is in contact with the patella during the usual loading process and because the thickness of the cartilage in this region is reportedly rather constant. In vivo single-impact trauma has been repeatedly shown to promote chondrocyte death in both in vivo and in vitro. Reductions in bovine chondrocyte viability are predominately in the superficial region of the cartilage, and as impact stress increases, cell viability decreases. In the present study, cell death was significantly higher in the superficial cartilage region than in other regions and in explants compressed to ≥60% strain than in those subjected to less compression. Consistent with these data, compression to ≥60% strain induced distinct regions of cell loss.

Chondrocyte cluster formation is a major characteristic of osteoarthritis. Clusters express markers of hypertrophy such as type X collagen, alkaline phosphatase, and osteocalcin as well as the matrix molecule fibronectin; however, the role of chondrocyte clusters in the progression of osteoarthritis remains largely unknown. With the model used in the present study, chondrocyte cluster formation was significantly affected by degree of strain and region. Cluster formation was significantly higher in the deep zone of all compressed specimens, compared with in the same region in control specimens, and in the middle zone of specimens compressed to ≥60% strain. However, only specimens compressed to 60% strain had significant increases in cluster formation in the superficial zone, compared with formation in control specimens. It is possible that compression to 70% or 80% strain generated localized stresses in the superficial region that prevented chondrocyte cluster formation or it could simply be that these high strains caused cell death, thereby preventing cluster formation. Interestingly, significant increases in chondrocyte cluster formation were observed in injured cartilage, even when there was little or no change in ECM composition. That finding suggested that although chondrocyte clusters are typically found in diseased or damaged cartilage at a time when there is a loss of ECM macromolecules, cluster formation may form in response to a factor that is independent of ECM degradation. The ability to reproducibly promote cluster formation with the in vitro model used in our study will allow for highly controlled investigations into the role these clusters play in the progression of osteoarthritis.

Although there was no significant difference in type II collagen content among injury groups, compression at 60% strain caused a significant decrease, compared with type II collagen content in control specimens. In a previous in vivo study, cartilage injury by single-impact compression, degradation of type II collagen was also detected; however, this degradation was concentrated around the impact site and extended through the full depth of the tissue. In that study, collagen content was evaluated at the earliest 84 days after injury. It is possible that had the in vitro model used in the present study been maintained longer than 28 days, we may have observed significant regional changes in type II collagen content.

Chondrocytes play the important role of producing and maintaining the ECM, including matrix repair after injury. Chondrocyte death independent of any other insult to the cartilage will eventually result in matrix loss. Single-impact trauma has been repeatedly shown to promote chondrocyte death in both in vivo and in vitro. Reductions in bovine chondrocyte viability are predominately in the superficial region of the cartilage, and as impact stress increases, cell viability decreases. In the present study, cell death was significantly higher in the superficial cartilage region than in other regions and in explants compressed to ≥60% strain than in those subjected to less compression. Consistent with these data, compression to ≥60% strain induced distinct regions of cell loss.
Like all in vitro experiments, the model developed in the present study lacked certain in vivo conditions, including the presence of additional tissue types, cytokines, and biomechanical loading. We believe our experiments are the first step toward developing an in vitro model for further evaluation in more defined conditions, including quantitative biochemical analysis of the response of cartilage to injury.

References

41. Reddi R, Mow VC, Zimny ML, et al. The ultrastructure and bio-


