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Altered mechanics of cartilage with osteoarthritis: human osteoarthritis and an experimental model of joint degeneration

By Lori A. Setton, Dawn M. Elliott and Van C. Mow*
Department of Biomedical Engineering, Duke University, Durham, NC 27708
**Departments of Mechanical Engineering and Orthopaedic Surgery, Columbia University, New York, NY 10032*

Summary

Objective: Studies of cartilage mechanics seek to determine the fundamental relationships between mechanical behavior and the composition and structure of healthy cartilage and to determine mechanisms for changes associated with degeneration.

Method: The mechanics of normal and osteoarthritic (OA) human articular cartilage are reviewed. Studies of the initiation and pathogenesis of cartilage degeneration in the anterior cruciate ligament transection (ACLT) model of joint instability are also presented.

Results: In human cartilage with OA, tensile, compressive and shear behaviors are dramatically altered. These changes present as decreases in the modulus or stiffness of OA cartilage in tension, compression and shear loading, and increases in the propensity to swell as compared to healthy cartilage. In the ACL transection model of OA, similar changes in the mechanics of cartilage have been observed. In addition, changes in structure, composition, and as metabolism consistent with human OA have been found. Deterioration of the collagen-proteoglycan solid network, which appears to be focused at the articular surface, has been the earliest cartilage changes in the model. It remains to be determined if the initial disruption of the cartilage surface is a direct result of mechanical forces or a product of altered chondrocyte activity.

Conclusions: These data and continued research using experimental models of OA provide a basis for our understanding of the pathogenesis and the time course of events in OA and will lead to the development of better procedures for disease intervention and treatment.

Key words: Cartilage mechanics, Anterior cruciate ligament transection, Joint instability, Osteoarthritis.

Introduction

Articular cartilage is the thin layer of deformable, load-bearing material which lines the bony ends of all diarthrodial joints. The primary functions of articular cartilage are to support and distribute forces generated during joint loading, to stabilize and guide joint motions and to contribute to joint lubrication [1]. When an external load is applied to a joint, articular cartilage will deform, resulting in increased joint contact areas and decreased contact stresses. Loading and deformation of cartilage will generate a combination of tensile, compressive and shear stresses in the material. The response of cartilage to these stresses is very specialized due to the tissue's unique composition and structural organization. The response of the tissue to an applied load will vary with time, giving rise to viscoelastic effects such as creep, stress relaxation and energy dissipation. These viscoelastic behaviors arise from both interstitial fluid flow through the porous-permeable solid matrix, and from physical interactions between the solid matrix constituents (e.g., collagen and proteoglycan). In a healthy joint, articular cartilage may provide for load-bearing, energy dissipation and joint lubrication with little or no signs of wear. With injury or degeneration related to osteoarthritis (OA), cartilage changes will occur which are associated with significant loss of mechanical function, with the potential to cause further progressive degeneration of cartilage.

Studies of cartilage mechanics have sought to determine the fundamental relationships between composition, structure and mechanical behavior of healthy cartilage in order to determine the mechanisms for functional change associated with degeneration and ultimately, repair. Studies of human cartilage have provided us with the basis for quantifying cartilage mechanics in the

Address correspondence to: Dr Lori A. Setton, Department of Biomedical Engineering, Duke University, Durham, NC 27708-0281, Tel.: (919) 660-5131; Fax: (919) 684-4488; E-mail: setton@duke.edu

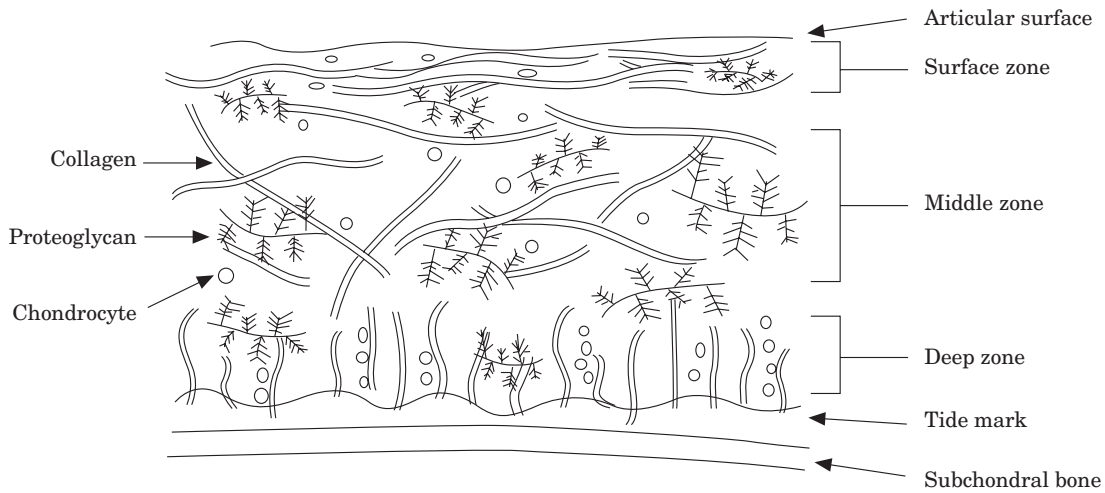

FIG. 1. Schema of articular cartilage. Structural variations with zone are depicted. In the surface zone, the collagen fibers are densely packed and oriented parallel to the articular surface; the proteoglycan content is low. In the middle zone, the collagen is more randomly oriented and the proteoglycan content is at a maximum. In the deep zone, the collagen fibers are larger and are oriented perpendicular to the subchondral bone, and the proteoglycan content is again low.

degenerative process, and will be briefly reviewed in this article. The initiation and pathogenesis of cartilage degeneration are frequently studied in experimental models, which allow for studies of the diagnosis, prevention and treatment of cartilage degeneration. In this article, we review one widely-studied experimental model of osteoarthritis, the anterior cruciate ligament transection (ACLT) model of joint instability. Changes in articular cartilage and meniscal function and their progression with time following ACLT will be reviewed. These and other studies of animal models of OA have provided the basis for our contemporary understanding of the pathogenesis of OA, and for ongoing and future studies of treatment options for the osteoarthritic degenerative process.

Composition and structure of articular cartilage

Articular cartilage consists of a composite organic matrix which is saturated with water (Fig. 1). The water phase of cartilage constitutes from 65–85% of the total tissue weight and is important in controlling many physical properties [1–5]. The dominant structural components of the solid matrix are the collagen molecules (~75% by dry tissue weight) and the negatively-charged proteoglycans (~20–25% by dry tissue weight). Collagen molecules, principally type II, assemble to form small fibrils and larger fibers with an orientation and dimension that vary throughout the depth of the cartilage layer [6–9]. The proteoglycans of articular cartilage are large polymers consisting of many aggregating macromolecules known as aggrecan [10–12]. A single aggrecan molecule consists of a protein core and numerous glycosaminoglycan side chains. Most aggrecan molecules are further bound to a single long chain of hyaluronan to form large proteoglycan aggregates of 50–100 $\times 10^6$ MW. The large size and complex structure of the proteoglycan aggregate function to immobilize and restrain it within the collagen network, thus forming the solid matrix of articular cartilage [1, 10]. The proteoglycans are negatively charged due to the presence of carboxyl and sulfate groups on the glycosaminoglycans, and so confer a net negative charge on the cartilage extracellular matrix. As a result, cartilage is highly hydrophilic, with a tendency to imbibe fluid, or swell, in order to maintain mechano–chemical equilibrium. This property significantly contributes to the mechanical function of articular cartilage by generating a large swelling pressure which facilitates load support and tissue recovery from deformations (see article in this issue by Mow and Hung). There are numerous other molecular species (e.g., types IV and IX collagen, biglycan, decorin) which contribute to the specialized function of articular cartilage [11–13].

The solid matrix of articular cartilage has a highly specific ultrastructure which may be divided into successive 'zones' from the articular surface to the subchondral bone (Fig. 1). Collagen fibers in the superficial-most zone of cartilage are densely packed and oriented parallel to the articular surface [6–9, 14]. This surface zone is also characterized by a relatively low proteoglycan

content and a low permeability to fluid flow [2, 15, 16]. In the middle or transitional zone, the collagen fibers are reported to be either random [17, 18] or radially oriented [9] and the proteoglycan content is at a maximum value for the tissue [2, 15, 19]. In the deep zone, adjacent to the zone of calcified cartilage and subchondral bone, the collagen fibers are larger and form bundles which are oriented perpendicular to the bone, and the proteoglycan content is again low [2, 8, 18, 19].

Pathological changes in cartilage function with osteoarthritis

In a healthy joint, articular cartilage may withstand the large forces associated with weight-bearing and joint motion over the lifetime of an individual. With osteoarthritis (OA), however, joint degeneration is characterized by cartilage and bony changes which lead to impaired joint motions, pain and disability [20]. Macroscopically, cartilage degeneration has been described as fibrillation of the articular surface, the presence of cracks or fissures, and by partial or complete loss of the tissue [21, 22]. Additional signs of OA include an increase in cartilage hydration, joint space narrowing, subchondral bone changes, osteophyte formation, evidence of altered cellular activity, and structural and compositional changes [2, 20–23]. A precise definition of OA is often difficult to achieve since presentation of OA in the human joint, and frequently in the animal joint, is rarely consistent. It is universally accepted, however, that major changes in cartilage function occur with OA which adversely affect the load-bearing, stabilizing and lubricating functions of the articular cartilage. An understanding of cartilage mechanics and associated changes with disease may be facilitated by considering the material behaviors in tension, compression, shear and swelling, as described here.

TENSION

When cartilage is loaded or stretched in tension, the collagen fibrils and entangled proteoglycan molecules align and stretch along the axis of loading. Resistance to tensile deformations and loads is generated principally by the intrinsic stiffness of the collagen fibers [24–27]. The tensile modulus (i.e., stiffness) and strength are measures of this resistance to tensile loading, and depend on the density and orientation of collagen fibers, and the type or amount of collagen cross-linking [28–31]. The tensile modulus reflects the intrinsic, or flow-independent, properties of the cartilage solid matrix when determined from stress–strain data at equilibrium or at very low strain-rates. The tensile modulus of healthy human articular cartilage has been found to vary from 5–25 MPa, depending on the location on the joint surface (i.e., high or low weight bearing regions), and the depth and orientation of the test specimen relative to the joint surface [28, 32]. With OA, the tensile modulus has been shown to decrease by as much as 90%, reflecting significant damage to the cartilage solid network [32]. Similarly, decreases in the tensile stiffness and fracture stress of cartilage have been reported for human cartilage with OA [25, 28]. These changes are a manifestation of the disorganized and disrupted collagen fibrillar network which has been observed macroscopically and histologically. Aging-related changes have also been reported, including a decrease in the tensile stiffness and fracture stress of articular cartilage [29, 33, 34] but are generally less severe than OA changes [32]. Aging changes may be attributed to alterations in collagen density or structure, which may be different from collagen changes associated with OA, such as fibril ‘unwinding’, fibrillation or loss of collagen cross-linking.

COMPRESSION

When cartilage is loaded in compression, volumetric changes and time-dependent viscoelastic behaviors occur due to fluid exudation from the tissue and fluid redistribution within the tissue [5, 35–37]. Exudation, imbibition, and flow of the interstitial fluid serve important roles in articular cartilage by contributing to load distribution [37], tissue recovery after load removal [35–38] and transport of large solutes in the cartilage [39]. The very low permeability of the cartilage matrix to fluid flow gives rise to very high fluid pressures and very high drag forces between the fluid and the solid matrix during interstitial fluid flow [5, 37, 40–42]. Fluid pressurization and energy dissipation generated through these fluid-solid interactions provide an efficient method to shield the cartilage solid matrix from stresses and strains associated with normal joint loading.

An important material property describing the flow-dependent behaviors for cartilage in compression is the hydraulic permeability (k), which is a measure of the matrix resistance to pressure-induced fluid flow. The hydraulic permeability is related to the solid matrix pore structure, proteoglycan concentration and water content [2, 5, 15, 36, 37, 40–43]. The permeability has been measured directly from the relationship between interstitial fluid flux and fluid pressure [5, 40–43], and from the

transient, flow-dependent strain or stress response in compression with use of an appropriate constitutive model [37]. At equilibrium, no fluid flow or pressure gradients exist within the tissue and the entire load will be borne by the solid matrix. Therefore, the compressive modulus measured at equilibrium will reflect the 'intrinsic' compressive stiffness of the cartilage solid matrix, whereas the modulus measured at other time points may erroneously represent the artifactual effects of interstitial fluid flow and fluid pressurization [37, 44, 45].

The compressive behavior of healthy human articular cartilage has been studied in uniaxial (i.e., confined compression) and indentation configurations [29, 38, 44–48]. Estimates of the compressive modulus were first recorded as the relationship between deformation and applied load at short and long times after loading [38, 44, 45]. Values for the compressive modulus of human articular cartilage varied from 2–10 MPa shortly after loading, with values of ~0.6 MPa at long times after loading [5, 38, 45]. In later studies, the compressive properties of cartilage were determined with use of a biphasic constitutive law which models cartilaginous tissues as interacting solid and fluid phases [37, 46, 47, 49, 50]. This theory permits simultaneous determinations of the hydraulic permeability (k) and compressive modulus and Poisson's ratio. Typical values of k for healthy human articular cartilage range from 0.5 to 8.0×10^{-15} m^4/N-sec [46] and compare well to values measured directly from permeation tests [5, 36, 40, 41]. Similarly, values for the compressive modulus vary from 0.4–2.0 MPa for human cartilage [46, 47] and compare well to values measured for long-term compressive moduli [5, 38, 44, 45]. The Poisson's ratio measured for human knee cartilage in compression is nearly zero, while that for most other species is significantly non-zero and up to 0.4. The hydraulic permeability has been shown to inversely correlate with the concentration of tissue glycosaminoglycan and to directly correlate with water content [15, 40, 43, 46], while the compressive modulus correlates inversely with water content [46]. These studies give evidence that the tissue's the negatively-charged proteoglycans and interstitial water have an important influence on both the intrinsic compressive stiffness and flow-dependent transient (i.e., kinetic) behaviors of human cartilage in compression.

Articular cartilage with surface fibrillation, pitting, or fraying has been shown to be more compliant, or deformable, in compression [38, 44, 48]. Armstrong and Mow [46] found the compressive modulus of human cartilage to decrease with increasing severity of degeneration as assessed using histochemical grading schemes or India-ink staining methods. There was also evidence of a decrease in the modulus with advancing age, which was difficult to isolate from possible degenerative effects. In contrast, the hydraulic permeability was not found to vary with either age or degeneration. Apparently, cartilage changes associated with OA, such as fibrillation, increased hydration, and decreased proteoglycan content, will act to compromise the intrinsic compressive stiffness of cartilage more severely than the flow-dependent behaviors [43].

SHEAR

Cartilage responds to shearing forces by both stretching and deformation of the solid matrix so that in pure shear, the tissue will deform with no change in volume, no pressure gradient, and no fluid flow through the matrix [51, 52]. Viscoelastic effects in shear are a result of frictional interactions between macromolecules of the solid matrix such as collagen and proteoglycan. Collagen fibers appear to be chiefly responsible for cartilage resistance to deformations or loading in shear, while the proteoglycans may contribute by generating a swelling pressure and network prestress which permits the matrix to more effectively resist shear [52]. Shear studies of cartilage have been performed to characterize the shear stiffness of the matrix at equilibrium, as well as the intrinsic or flow-independent viscoelastic behaviors of cartilage under transient or dynamic conditions [44, 51–55]. Important material properties for cartilage behavior in shear include the equilibrium modulus (μ), a measure of the intrinsic matrix shear stiffness at equilibrium, and the dynamic modulus (G*), a measure of combined elastic and viscous effects when cartilage is subjected to dynamic shearing. Also, the loss angle (δ) is a measure of the dissipation associated with viscoelastic effects and can range from 0° for a perfectly elastic solid to 90° for a perfectly viscous fluid.

Few shear studies have been performed on human articular cartilage and the bulk of our knowledge of cartilage shear behavior is due to testing of bovine and other animal tissues. For healthy bovine or canine cartilage, values of the equilibrium shear modulus (μ) have been reported to vary from 0.1–0.4 MPa whereas values for |G*| are in the range of 0.2–2.0 MPa and vary with frequency [52–55]. Values for δ are in the range of 9–15° demonstrating that cartilage behavior is similar to that of a viscoelastic solid material. In

an early study, Hayes and Mockros [44] obtained a measure of the equilibrium shear modulus of 2.6 MPa for normal human articular cartilage from torsional shear testing. Degenerate cartilage was found to be significantly more compliant in shear, which was attributed to fibrillation of the articular surface and loss of 'ground substance' with OA. The changes observed in the shear behavior of human OA cartilage are consistent with trends demonstrated for bovine cartilage depleted of glycosaminoglycans [51, 52] although more studies are required to fully determine the mechanisms for change in shear mechanics of cartilage with OA.

SWELLING

Swelling in cartilage arises from the presence of a high density of negatively-charged proteoglycan molecules (for a more detailed discussion, see paper by Mow and Hung of this volume). Each proteoglycan-associated negative charge requires a mobile counter-ion (e.g., Na+) to maintain electroneutrality, giving rise to an imbalance of mobile ion concentrations between the tissue and the external solution and a resultant swelling pressure [40, 56–58]. These ionic interactions are modulated by the counter-ion concentration so that the swelling pressure of cartilage will decrease with increasing interstitial ion concentrations due to 'charge-shielding' of the proteoglycan molecules. This swelling pressure is balanced by the stresses generated within the collagen-proteoglycan solid matrix, and by stresses occurring during joint loading [56, 58]. Therefore, at equilibrium and physiological concentrations, the solid matrix of cartilage is in a state of pre-stress. These states of pre-stress are important for the tissue's load-bearing functions since pre-stress will strongly affect stress distributions within the tissue during loading [56–60].

The balance between swelling pressure and matrix stresses will determine the dimensions and hydration of articular cartilage when unloaded. For example, articular cartilage has long been observed to swell or imbibe water in hypotonic salt solution, and to shrink or lose water in hypertonic salt solutions [61–63]. Quantification of water imbibition after equilibration in an ion bath has been used extensively as a technique to study cartilage swelling with degeneration [2, 64–67]. In general, swelling effects recorded as tissue weight change have been described as minimal for healthy human articular cartilage (<3% in 0.015 M as compared to 0.15 M NaCl) but significant (up to 30% water weight gain) for fibrillated or OA human cartilage. Indeed, an important characteristic of degenerate human cartilage is its propensity to imbibe fluid to a greater extent than normal cartilage, as reported in numerous independent studies [3, 64–70]. This water weight gain is believed to reflect damage to the cartilage fibrillar network which will restrain cartilage swelling in healthy tissue. With aging, there is evidence of no change or even a slight decrease in cartilage hydration, so that swelling may be one of the few distinguishing characteristics between OA and aging [65, 70].

The swelling behaviors of cartilaginous tissues have also been studied in isometric tensile and compression swelling experiments [61, 63, 68, 69, 71, 72]. In these tests, samples of cartilage are held at a fixed length and subjected to a change in the ionic environment of the bathing solution. The force response will decrease with a solution change to a hypertonic bath reflecting a decrease in the interstitial swelling pressure. Important material properties for cartilage in isometric swelling are the ratio of isometric stresses in two different osmotic environments, as well as the time constant of stress decay which is proportional to the ionic diffusion coefficient within the tissue. The isometric swelling behavior of normal and degenerate human articular cartilage has been studied in isometric tension by Akizuki and co-workers [68]. Both the equilibrium and transient swelling behaviors were found to relate to the ratio of collagen to proteoglycan, and to proteoglycan concentration alone. These results support the prevailing hypothesis that maintenance of the integrity of the collagen and proteoglycan network in cartilage is important in governing the normal swelling behaviors of articular cartilage.

Anterior cruciate ligament transection model of osteoarthritis

Studies of the mechanical behaviors of cartilage have advanced our understanding of the pathology of human OA. They provide little information, however, on the temporal progression of degeneration particularly at the earliest stages of the disease. Studies of experimentally-induced cartilage degeneration in animal models have been pursued because they provide a means to track the sequence of early events which occur during cartilage degeneration [73, 74]. In addition, they provide an important tool to isolate the OA degenerative process from aging, which has been a major problem in studies on the etiology of human

OA. Many experimental models of cartilage degeneration have been based on altered joint mechanics, such as impact loading or damage to the ligamentous or meniscal tissues [73, 74]. In the models of damage to the ligaments or menisci, inhibition of these force-attenuating tissues alter both the magnitude and distribution of joint forces which are applied to the cartilage surface *in vivo*. Mechanical instability, therefore, is believed to be the primary initiating factor for the onset, and possibly also the progression of cartilage degeneration in human OA.

The anterior cruciate ligament transection ACLT model for joint instability and OA has been the most widely used model for the study of degenerative changes in articular cartilage [e.g., 53, 76–99]. ACLT produces increased anterior drawer in the knee joint at extension and at 90° of flexion, as well as an increased internal rotation [75, 76]. Following ACLT, morphological and histological changes in the cartilage occur, including fibrillation or roughening of the articular surface, early loss of proteoglycan and collagen fibril organization, increased cellularity, meniscal changes, joint capsule thickening and osteophyte formation [77–83]. Also, biochemical and metabolic cartilage changes have been reported, including an increase in hydration, increase in proteoglycan and collagen synthesis and breakdown [76, 83–90], and alterations in proteoglycan molecular structure [82, 86, 89, 91]. Many of these changes occur within the first three to six weeks after the surgery, and are potential sources for the observed changes in cartilage mechanical function. Finally, there is evidence that cartilage changes in the canine knee following ACLT will progress with time and exhibit characteristics similar to those observed in end-stage human OA [92].

From studies of human articular cartilage, we know that OA produces compromised mechanical properties for cartilage in tension, compression and shear. We know that these changes reflect a damaged cartilage fibrillar network which is also responsible for the elevated water content observed in swelling studies of human articular cartilage. What remains to be determined from studies of experimental models of OA such as the ACLT model, are the sites for initiation of cartilage degeneration in the joint; are they in weight-bearing or non-weight-bearing sites; are they at the articular surface or deeper zones? We also seek to determine which characteristics of mechanical function are compromised first (e.g., compressive or tensile properties?), what are the underlying compositional and structural mechanisms for this change of mechanical properties, and presumably function, and how are they interrelated in the progression of disease? Ultimately, we seek to determine if pharmaceutical or surgical strategies can serve to inhibit or reverse cartilage degeneration with OA, and at what time points is medical intervention most appropriate and successful. Studies of cartilage mechanics in the ACLT model of OA have been designed to address some of these questions, with findings that are summarized in the next sections.

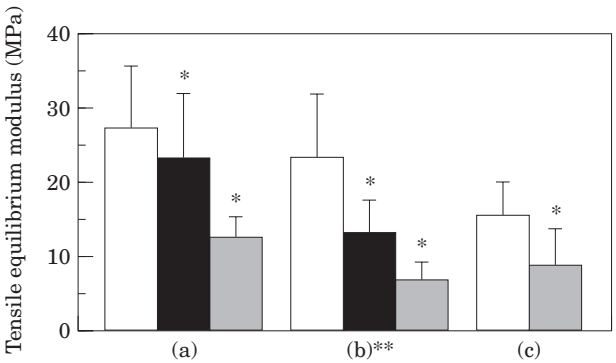

FIG. 2. Equilibrium tensile modulus, E, for articular cartilage in a canine model of OA. Times are weeks after ACLT in the (a) greyhound femoral groove and (b) femoral condyle (Setton *et al*., 1994) and in the (c) beagle femoral condyle (Guilak *et al*., 1994) (data shown as mean and standard deviation: *different from control, $P<0.05$ MANOVA; **different from femoral groove, $P<0.05$, Student–Newman–Keuls). □, control; ■, 6 weeks; □, 12 weeks (a, b) 16 weeks (c).

TENSION

In an early study of ACLT in the canine knee, decreases in the equilibrium tensile modulus of articular cartilage were observed as early as three weeks after surgery [98]. More recently, we studied the tensile behavior of canine cartilage at six and twelve weeks following ACLT in greyhounds, and at sixteen weeks following surgery in beagles [95, 96] (Fig. 2). In general, ACLT resulted in a significant decrease in the tensile modulus of femoral condylar cartilage prepared from the articular surface. Values for the reduction in tensile modulus were between 40–65% at the later time periods after surgery for both animal models. These changes were consistent for differences in surgical procedure (i.e., open arthotomy vs. arthroscopy), sites of cartilage testing (i.e., medial vs. lateral femoral condyle), and for different control populations (i.e., age-matched controls. vs. contralateral controls), demonstrating the repeatability of instability-induced degenerative changes in the canine ACLT model of OA. In the greyhound model, reductions in the tensile modulus were

progressive from a decrease of ~28% at six weeks to a 64% decrease at twelve weeks after surgery [95]. Also, reductions in the tensile modulus of cartilage from the femoral condyles were of similar magnitude to those of cartilage from the femoral groove, with similar findings observed at all sites in both patello-femoral and tibio-femoral articulations. These findings demonstrate that the response of cartilage to ACLT does not have a strong dependence on contact or weight-bearing areas in the joint. Importantly, the trend and magnitude of the findings for a reduced tensile modulus for articular cartilage are similar to findings for human articular cartilage with fibrillation or osteoarthritis [32].

In these studies of the canine ACLT model of OA, the biochemical composition of site-matched cartilage has also been quantified to test for potential relationships between changes in cartilage constituents and the observed loss of tensile stiffness [89, 95, 96]. Cartilage was found to have a significantly higher hydration and lower collagen and glycosaminoglycan contents per wet weight after ACLT. Again, these changes were similar for cartilage from different sites on the distal femur. These changes reflect the higher water content observed in the degenerate cartilage, as few changes in collagen or glycosaminoglycan contents were observed on a dry weight basis [89, 96]. The density of hydroxypyridinium cross-links in this model has also been studied, as these cross-links serve to covalently stabilize intrafibrillar and interfibrillar interactions between collagen molecules and so are an important measure of the collagen macromolecular structure [96]. There was evidence of an 11% decrease in hydroxypyridinium crosslink content on a collagen basis which demonstrates that collagen may be undergoing structural changes in this model. Together with findings for elevated collagen synthesis and degradation in the ACLT model [84, 85], these findings indicate that there is an accelerated turnover of the collagen network at the articular surface. These compositional changes in the water, collagen and glycosaminoglycan contents, and structural changes in the collagen molecule are consistent with findings for human osteoarthritic cartilage. The absence of powerful correlations between tensile stiffness and collagen content or cross-link density, however, suggests that collagen fibrillar organization rather than composition is dominant in governing the tensile properties of healthy and degenerate articular cartilage, particularly at the articular surface.

In a related study of the greyhound model, no changes were observed in the tensile modulus of the medial or lateral meniscus following ACLT at either 6 or 12 weeks after surgery [99]. Gross changes in the morphology and shape of the meniscus were observed, however, including fibrillar disorganization and tearing, and cellular division. There was an increased water content at 6 and 12 weeks and a decreased glycosaminoglycan content at 6 weeks, although no intrinsic changes in the tensile material properties appear to result. There were no significant changes in hydroxyproline content or hydroxyproline crosslink density at either time point. Differences in the response of cartilage and meniscal tissues to ACLT may partly reflect altered metabolic activities in these tissues. Elevated catabolic and anabolic responses have been observed in the articular cartilage of the canine model of ACLT [76, 83–90], which suggests one mechanism for the deleterious effect of joint instability on cartilage, but perhaps not meniscus during the first 12 weeks of this model of OA.

COMPRESSION

In an early study of the canine ACLT model, decreases in the compressive modulus of articular cartilage were observed at some sites on the tibial plateau at both early (3 weeks) and later times (12–23 weeks) after surgery [98]. These findings were later corroborated by studies of the compressive behavior of cartilage after ACLT in the greyhound model using an indentation test and the biphasic theory [95]. The compressive modulus of articular cartilage was found to decrease independent of site on the medial tibial plateau beginning at six weeks after surgery (Fig. 3). The magnitude of this decrease averaged 25% at the later time point after surgery. Furthermore, there was evidence of a significant increase in the hydraulic permeability of tibial cartilage of ~48% at twelve weeks after surgery (Fig. 4). More recently, a study of the uniaxial compressive behavior of cartilage in a rabbit model of ACL transection provides partial corroboration of these findings [97]. The compressive modulus of rabbit femoral cartilage was found to decrease by ~18% at nine weeks after ACLT with no evidence of a change in the hydraulic permeability. This loss of compressive stiffness suggests a matrix which is more compliant and deformable in compression, consistent with changes observed in human cartilage degeneration and OA. The increase in permeability of tibial cartilage observed in the canine model is likely to be one of the more injurious changes in the mechanics of articular cartilage after ACLT. Elevated permeabilities produce increased matrix deformations and decreased

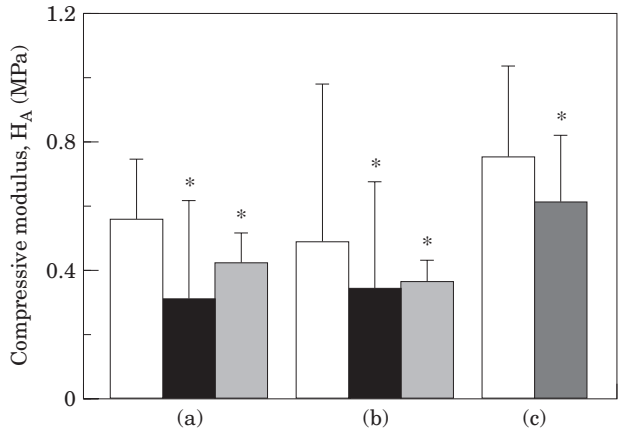

FIG. 3. Equilibrium compressive modulus, H_A, for articular cartilage in the ACLT model of OA for two different species. Cartilage was tested at sites (a) covered and (b) uncovered by the meniscus *in situ* in the greyhound knee (Setton *et al.*, 1994) and on the (c) medial femoral condyle in the rabbit knee (Sah *et al.*, 1997) (data shown as mean and standard deviation: *different from control, $P<0.05$ MANOVA). □, control; ■, 6 weeks; ▩, 9 weeks, □, 12 weeks.

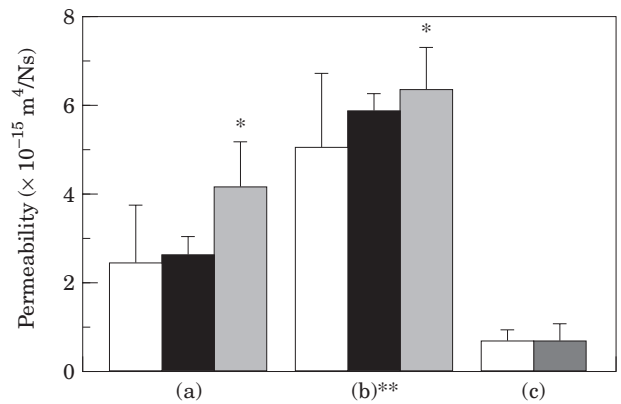

FIG. 4. Permeability, k, for articular cartilage in the ACLT model of OA for two different species. Cartilage was tested at sites (a) covered and (b) uncovered by the meniscus in situ in the greyhound knee (Setton *et al.*, 1994) and on the (c) medial femoral condyle in the rabbit knee (Sah *et al.*, 1997) (data shown as mean and standard deviation: *different from control, $P<0.05$ MANOVA; **different from covered, $P<0.05$, Student–Newman–Keuls). □, control; ■, 6 weeks; ▩, 9 weeks, □, 12 weeks.

hydrostatic pressures under physiological loading [1, 16]. These changes will be associated with elevated fluid flow velocities and exudation, and reductions in fluid pressurization as a mechanism for load support in the degenerate cartilage [1].

A significant and direct correlation was observed between the compressive modulus and glycosaminoglycan content per wet weight in the femoral cartilage in the rabbit, as well as an inverse correlation between compressive modulus and water content for both tibial and femoral

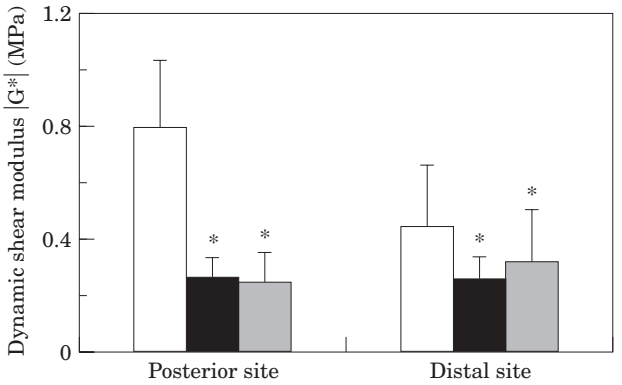

FIG. 5. Dynamic shear modulus, |G*|, for canine articular cartilage in the ACLT model of OA (Setton *et al.*, 1995). (data shown as mean and standard deviation: *different from control, $P<0.05$ MANOVA). □, control; ■, 6 weeks; □, 12 weeks.

cartilage in the rabbit and canine knees [95, 97]. Importantly, decreases in hydraulic permeability were directly correlated to increases in hydration for the canine cartilage [95], suggesting that elevated water content is responsible for the compromise in both the transient (i.e., governed by the permeability, k) and equilibrium compressive properties (i.e., H_A) of degenerate cartilage. The observed relationships between compositional measures such as glycosaminoglycan and water contents and compressive material properties for the ACLT model are consistent with our understanding of human cartilage mechanics. With cartilage degeneration following ACLT, however, we have further established that loss of compressive stiffness for the solid network is an early event and may precede changes in the transient, flow-dependent compressive behavior as detected by changes in the hydraulic permeability.

SHEAR

In the canine ACLT model of OA, the mechanical behaviors of medial femoral condyle cartilage were studied in torsional shear testing [53]. Results indicate that the equilibrium shear modulus (μ) for cartilage from the medial femoral condyle decreased by an average of 65% at six weeks after surgery with no further progression from six to twelve weeks. This response to ACLT was found to be independent of site on the femoral condyle. Also, this trend is consistent with that observed for human cartilage with fibrillation in shear testing [44]. There was evidence of a large decrease in the magnitude of the dynamic shear modulus (|G*|) at six weeks after surgery (average ~45%; Fig. 5), with little evidence of a change

from six to twelve weeks. In measures of viscous dissipation, however, there was evidence of an increase in the loss angle (δ) of ∼35% at twelve weeks after surgery. These changes in the shear behavior do not directly reflect transient, flow-dependent phenomena as there are no volumetric changes in torsional shear testing. Therefore, these changes in the equilibrium and dynamic shear behaviors of cartilage following ACLT reflect changes to the collagen-proteoglycan solid network and point to increased inter-molecular frictional dissipation in the experimental cartilage (i.e., loosening of the collagen-proteoglycan solid matrix).

The hydration of all femoral condyle cartilage increased after ACLT, a finding which was inversely correlated to the decrease in dynamic shear modulus [53]. This dependence of the shear behavior on hydration likely reflects an altered pre-stress state for the degenerate cartilage, as this tissue was found to be more swollen and hence, more extended prior to shearing [52, 53]. In summary, the observed changes in shear behavior support the concept of a disruption in the collagen-proteoglycan matrix that directly affects the shear and tensile behaviors of degenerate articular cartilage, and indirectly the hydration through the swelling mechanism (also see next section).

SWELLING

Studies measuring water weight gain in articular cartilage after ACLT have uniformly reported increased hydration when compared to controls [53, 78, 89, 93, 95–97]. In addition, we have studied the swelling behaviors of cartilage following ACLT in the isometric tensile swelling experiment [95, 96]. Consistent findings of an increase in the ion-induced stress-relaxation were observed (∼48% at twelve weeks after surgery in the greyhound model; ∼67% at sixteen weeks after surgery in the beagle model), a parameter which reflects the equilibrium tensile stress in physiological saline relative to distilled water. This increased stress-relaxation response was directly correlated to tissue hydration, and inversely to collagen cross-link density and measures of glycosaminoglycan and collagen contents on a wet weight basis. These findings for elevated hydration and stress-relaxation in the experimental cartilage are consistent with the concept that disruption of the solid network will inhibit the ability of the tissue to resist swelling, as observed for human degenerate and OA cartilage. A balance of swelling pressure with collagen network stress seems to be the determining factor for tissue hydration and swelling [56]. The decreased tensile and shear stiffnesses of cartilage following ACLT, together with an elevated collagen cross-link density, all point towards a disruption in the integrity of the collagen network as the initiating event for changes in the cartilage swelling behavior.

Discussion

Precise quantitation of cartilage degeneration and its exact relationship to changes in the mechanical behavior and function in diarthrodial joints remains an elusive topic of study. While there is general agreement between changes of the mechanical properties of human OA cartilage with those from animal ACLT OA models, there are fine differences which reflect possible differences between human knee cartilage and the knee cartilage from various species used in the animal models of OA. Nevertheless, the experimental models of OA with ACLT have been successful in recreating many of the cartilage changes associated with OA, and so provide a basis for studying the sequence of events during the progression of early articular cartilage degeneration. In both human OA and experimental models of joint instability, the earliest pathological changes in cartilage are the deterioration of the cartilage solid matrix, which appears to be focused at the articular surface. This disruption in matrix integrity will involve a disruption of the normal mechanical stress states for the entire tissue, and is at least partly responsible for the elevated swelling propensity in the degenerate cartilage. The presence of a stiff and tightly woven surface region in articular cartilage has been shown to govern the permeability of the surface zone [15, 16] and is required to restrict fluid flow and to contain the swelling pressures generated by the negatively charged proteoglycans [56]. Damage to the cartilage solid matrix is therefore associated with dramatic changes in cartilage mechanics, including a significant loss of the tensile, compressive and shear stiffnesses, as well as a significant elevation in the hydraulic permeability of the cartilage layer [1]. While many of these studies point to disruption of the collagen-proteoglycan solid matrix as the initiating factor for progressive degeneration, it remains to be determined if this initial disruption is a direct result of mechanical forces or a product of altered chondrocyte activities, such as elevated matrix catabolism. Indeed, decreases in collagen content and altered collagen cross-linking were found to contribute to the

decreased tensile stiffness of canine cartilage following ACLT. Furthermore, the absence of site-specific responses to the mechanical instability induced by ACLT suggests that metabolic events at the joint level may also be a key factor in the progression of the disease, even at early times after onset of the damage. Developing a clear understanding of the time course of events in OA, in part through the use of experimental animal models such as ACLT, is critical at this time in our research.

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