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The acute structural changes of loaded articular cartilage following meniscectomy or ACL-transection

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Summary

Objective: Meniscectomy and anterior cruciate ligament (ACL) rupture have been identified as precursors of osteoarthrosis (OA) in clinical reviews and animal experiments. In this study, the acute effects of these injuries on articular cartilage matrix deformation, preserved in a loaded state using a cryopreservation technique, were studied by scanning electron microscopy (SEM).

Method: Whole knee joints from adult White New Zealand rabbits (N=87) were loaded ex vivo, using a simulated quadriceps pull under static and cyclic loading conditions, following medial meniscectomy or transection of the ACL. Specimens were plunge-frozen while under load, or following a recovery period, and prepared for SEM by cryofixation. Using SEM and photographic images, the medial tibial plateau cartilage was assessed both qualitatively and quantitatively.

Results: After meniscectomy, significantly increased bending and crimping of radial collagen fibers occurred with static loading. Compared to intact knees, the area of tibial cartilage showing an indentation was increased by 80% (P<0.05), the articular cartilage thickness was significantly more reduced when under load (for high force long duration static loading, intact joints had 53%±3 reduction in cartilage thickness compared to 39%±4 after meniscectomy, P<0.05), and it took nearly twice as long for the cartilage thickness to recover following loading. These post-meniscectomy differences were either not present or were minimal when the joint was allowed to extend when loaded. ACL-transection slightly increased collagen deformation in the deeper zones, but only with cyclic loading.

Conclusion: The findings indicate that, with static loading, significantly increased deformation of articular cartilage collagen structure can occur following meniscectomy, but is minimized by joint motion. This increased deformation may be relevant to the etiology and progression of joint degeneration. © 2000 OsteoArthritis Research Society International

Key words: Osteoarthritis, Meniscectomy, ACL-transection, Collagen structure, Scanning electron microscopy, Overloading, Stress.

Introduction

Meniscal and anterior cruciate ligament (ACL) injuries are common in clinical practice. Meniscectomy, a standard treatment for unstable meniscal tears, is associated with the later development of osteoarthrosis (OA). An increased risk of developing OA also has been described for the rupture of the ACL, although the relationship is not as clear. Both lesions are followed by typical degenerative changes in the articular cartilage of experimental animal models.

The sequence of factors leading to this degeneration is unknown. Within the first weeks after meniscectomy or ACL-transection, the metabolic activity in and composition of articular cartilage change. Clearly, the biomechanical environment of the joint is disrupted. Most notably, medial meniscectomy increases average stresses in the contact regions due to decreased tibio-femoral congruency. ACL insufficiency may increase muscle forces required to stabilize the joint. These increased loads may exceed the mechanical capacity of the cartilage collagen matrix. Ultrastructural studies have shown that abnormalities of the collagen matrix are a consistent feature of experimental and clinical OA. Changes such as collagen crimping, swelling and fissuring of the collagen fiber matrix have been observed. Such findings have led to the theory that mechanical failure of the matrix is the primary causal factor in degenerative joint disease. However, human knees may show no evidence of degeneration within the first years after meniscal surgery/ injury or ligament rupture, raising the question of whether factors such as direct damage to the cartilage at the time of injury are responsible.

To date, little is known about the immediate effects on articular cartilage structure in a joint were the meniscus has been removed or the ACL been transected, because until recently it has been impossible to study the collagen structure of loaded joints. We have developed a technique by which whole knee joints can be loaded, either statically or dynamically, and then preserved in the loaded configuration by plunge-freezing and freeze-substitution. Initial results with this technique showed that deformation of the collagen matrix of...
rabbit tibial plateau cartilage corresponds to load magnitude and duration, and that meniscectomy produces larger deformations.36,37 In this study, the effects of joint loading on cartilage collagen structure are analysed morphologically and quantitatively following removal of the meniscus or transection of the ACL. The intention is to determine whether the observed deformation of the plateau following these lesions is measurably different from loaded cartilage whether the observed deformation of the plateau following these lesions is measurably different from loaded cartilage.

Materials and methods

SPECIMEN PREPARATION

Eighty-seven adult female New Zealand White rabbits (mean body weight=4.1 kg, age=11–13 months) were killed and both knee joints were isolated at the proximal femur and the distal tibia. Soft tissues were removed from each knee, with care taken not to damage the knee ligaments or joint capsule. Radiographs were examined for assessment of skeletal maturity and to exclude joints with deformities. The knee joints were loaded (1) intact, (2) with the medial meniscus totally excised, or (3) with the ACL transected. The meniscus was removed by detachment of the anterior and posterior horns from their tibial attachments and mobilization from the intact collateral ligament. The ACL was transected in the middle through a 6–8 mm parapatellar capsular incision, closed by continuous suture. Testing of the specimen was performed within 30 min of donor death.

MECHANICAL LOADING

A simple loading device for static and cyclic load application was used for ex vivo testing of rabbit whole knee joints and has been described previously.36,37 The load was applied as a simulated quadriceps force applied by means of a wire looped through a drill hole in the proximal patella. Tension on the wire was generated by a material testing machine (Mikrotron 654, Russenberger and Müller Ltd, Schaffhausen, Switzerland) under displacement control with the magnitude of the force set with the aid of a load cell. For static loading, knee extension was limited, held at 90° of flexion (mid-range of motion). High or low loads were applied as quadriceps forces of 3× or 1× (high or low) body weight. Duration of loading was either long or short (30 min or 5 min). To simulate motion (cyclic forces), the knees were permitted to extend from 70° to 150° at 1 Hz for 30 min. Extension was resisted by a spring, generating sinusoidal cyclic extension forces, reaching peak forces of 3× and 1× body weight. While loading, all specimens were submerged in isotonic saline solution (pH 7.4 buffered Ringer’s solution at room temperature).

For each joint condition (intact, meniscectomized, ACL transected) and loading situation (N=5) knee samples were used (ACL-transsected samples were tested only at 3× body-weight loading). Non-loaded intact joints were used as controls (N=10). To determine the rate of recovery, groups of N=4 intact and meniscectomy joints were unloaded and held at 90° flexion for load-free periods of either 2, 4, 16 or 30 min prior to cryofixation (this was performed for samples after 30 min of static loading at 3× and 1× body weight or cyclic loading at 3× body weight). Recovery was not analysed for the ACL-transsected knee joints as no deformation differences with respect to intact joints were observed for static loading.

TISSUE PRESERVATION

A previously developed protocol36,39 was used for preservation of the knee joints. Immediately after completion of the loading procedure, the joints were frozen while under load (motion was discontinued for cyclic loaded joints) by immersion into isopentane slush, pre-cooled with liquid nitrogen to below −160°C for 4 min, removed from the loading device and fixed by freeze-substitution. Then the joints were carefully opened and the tibial plateau, together with approximately 1 mm of subchondral bone, was excised after manual sawing. Prior to photographing and measuring the indentation in the tibial plateau, the meniscus was removed. Afterwards, the plateau was fractured in a plane perpendicular to the articular surface and through the middle of the indentation (from medial to lateral) in the cartilage surface created by contact with the femoral condyle.40 The samples were critical-point dried (POLARON E3000 critical point drier, Agar Scientific Ltd, Essex, U.K.) and examined with an SEM (Hitachi S-4100 field emission SEM, Hitachi Ltd, Tokyo, Japan). The images were collected digitally with an image management system (Quartz PCI, Quartz Imaging Corporation, Vancouver, Canada).

An additional six intact loaded knee joints were prepared for light microscopy (static and cyclic high force, long duration loading and unloaded; two samples each). The samples were loaded, frozen and fixed as described above, rinsed in 100% ethanol and placed in 100% xylol before infiltration and embedding in methyl-methacrylate. Sections were cut to 200 μm thickness in the frontal plane with a microtome (Sawing microtome 1601, Wetzlar, Germany) and polished (Polishmachine Stähli-Lapp, Stähli AG, Biel, Switzerland) to 80 μm thickness and stained with Azan to highlight the collagen fibers.41

EVALUATION

The femoral condyle indents the tibial plateau when the knee is loaded (Fig. 1). The tibial plateau surface and these indentations were initially examined under a photomicroscope. More detailed morphological observations were made with light microscopy and especially with SEM. We have previously shown that the straight radial collagen fiber bundles in the rabbit tibial plateau develop an acute bend and sinusoidal crimp where the loaded plateau is indented by the femoral condyle. The general morphological features of the bending and crimping patterns, as well as cleft formation in the cartilage surface and the appearance of the condylar indentation produced by the loading were compared as previously described.

Quantitative measurements were also performed as described previously.37 Before cryo fracturing of the plateau, the area of tibial indentation (area of tibial cartilage showing an indentation) was measured. The deformation of the collagen in the tibial plateau was quantified from the freeze-fractured samples. The minimum cartilage thickness after loading, the level (height) of the acute bend in the radial collagen fibers, and the period of collagen fiber crimping was measured on SEM images utilizing an image analysis and measurement program (PC-Image, Foster Findlay, Newcaste, U.K.). Only fractures through the middle of the indentation of the tibia plateau were used for quantitative evaluation. The parameters of indentation area and cartilage thickness were also used for analysis of visible cartilage recovery.

The cartilage surface was viewed under a stereomicroscope (Wild, Heerbrugg, Switzerland) and examined at a
magnification of ×12.5 while immersed in 100% ethanol, to prevent drying effects. The margin of the indentation (Fig. 1) was traced on a digitizing table (Kontron Electronics, Munich, Germany) by projecting the cursor onto the field of view. The area (mm²) was then calculated and recorded.

The minimum cartilage thickness (distance from the surface to the subchondral bone, perpendicular to the surface) at the center of each indentation in the tibial plateau was measured from calibrated SEM images of the loaded specimens. In addition, the unloaded cartilage thickness was derived from measurements of the total collagen fiber length (from the calcified cartilage/deep zone interface to the articular surface) at the same location, and the loaded cartilage thickness was then expressed as a percentage of calculated unloaded thickness. To validate this measure of unloaded cartilage thickness, the difference between the length of a collagen fiber and the corresponding cartilage thickness in unloaded control samples was compared with a paired t-test.

The height/level at which the acute bend of a collagen fiber at the center of maximum indentation occurred was quantified by measuring the distance from the subchondral bone to the bend and the entire length of the same fiber at that location. The level of the acute bend in loaded collagen fibers was expressed as a percentage of total fiber length. These measurements, as well as the thickness measurements, could be taken only from specimens where the entire fiber ran in the plane of the fracture.

For the control variables of loading mode and load magnitude, Q–Q-plots and Tukey–Anscombe plots were examined for normality. Main effects and interaction terms were evaluated by multiple regression analysis. All comparisons were analysed using the Ryan-Einot-Gabriel-Welch (REGQW) multiple range test at a significance level of P=0.05. Comparative analyses were carried out for the following three outcome variables: area of indentation, minimum thickness after loading and height of the acute bending angle. The maximum fiber crimp wavelength in the center of the indentation was measured (μm). No statistical tests were performed for fiber crimp comparisons.

**Results**

Macroscopic indentations from tibio-femoral contact were visible in all loaded tibial plateaus. In comparison to intact and ACL-deficient specimens, the indentations were visibly larger in those from which the medial meniscus had been removed for all static loading conditions (Fig. 1). These indentations grew into regions that had been previously covered by the meniscus. Isolated clefts were visible in the cartilage surface with all loading conditions for all joints (Fig. 1). No surface damage, such as fibrillation, was observed under the photomacroscope or the SEM. The unloaded samples exhibited no deformation of the straight collagen fibers that run vertically from the calcified cartilage to the surface (Fig. 2).

**INTACT JOINT**

Under static loading, the maximum fiber bending was observed in the center of the indentation but there was also significant deformation under the meniscus [Fig. 3(a)]. Low force long duration loading showed fibers oriented at an acute angle to the surface and no deformation in the radial zone [Fig. 4(a)]. After high force, long duration static load in the intact loaded joint, a newly formed tangential zone of collagen fibers lying almost parallel to the surface was visible in all loaded tibial plateaus.
observed in the superficial third of the cartilage thickness. The fibers of the deeper zone were tilted and did not completely retain their radial orientation [Fig. 5(a)]. Following all long duration static loads, segments of regular, sinusoidal crimping were seen in collagen fibers within the area of indentation. This was also found in light microscopy.

MEDIAL MENISCETOMY

The collagen fibers were less deformed in the outside areas following meniscectomy compared to the intact joints [Fig. 3(a, b)]. In the center of the tibial plateau the collagen structure within the condylar indentation was consistently more deformed under static loading, when meniscectomy was performed. With low static loads, the deformation was limited to the upper zones of the cartilage but involved a greater portion of the fiber length at the indentation center and was observed over a wider area under the condyle [Figs 3(b) and 4(b)]. Following high force, long duration static loads the full length of the collagen fiber was turned to lie parallel or almost parallel to the surface and pointed towards the periphery of the tibial plateau [Fig. 5(b)]. With motion (cyclic loading), not much difference in deformation was observed between the meniscectomized and intact joints. Both showed a superficial deformation that was limited to the tangential and transitional zone [Fig. 6(a)]. With all static loads, regions of crimping along the collagen fibers were more extensive in the meniscectomized knees than in the intact joints but were not present in joints moved during loading, i.e. cyclic loads [Fig. 4(c)]. In Fig. 7 the deformation characteristics of collagen fibers in intact and altered joint situations are summarized.

For all static loads, the area of tibial indentation (indentation created by the femoral condyle) was significantly larger \([P<0.05]\) in the meniscectomized joints when compared to the intact joint (Fig. 8). After low force and long duration the indentation was nearly 80\% larger following meniscectomy (16.6 mm\(^2\)±0.6 vs 9.4 mm\(^2\)±0.8, \(P<0.05\)). For high force cyclic loads, there was no difference between meniscectomized and intact joints. With low force there was a slight difference (9.0 mm\(^2\)±0.6 vs 7.0 mm\(^2\)±1.5, \(P<0.05\)). For both intact and meniscectomized joints, the indentation was not visible after a 4-min recovery period after cyclic loading. An indentation was still visible 16 min after low force and long duration static load in joints from which the meniscus had been removed (7.4 mm\(^2\)±0.4), but the intact joint had recovered completely by that point.

Meniscectomy was found to cause significantly greater thickness reduction under static loading conditions \((P<0.05)\) (Fig. 9). After low force and long duration static...
loads, the minimum thickness was 67.4% (±2.7) of resting height in the meniscectomized joints and 79.2% (±3.1) in the intact joint. The larger and longer loads resulted in the same decreased cartilage thickness for meniscectomized and intact joints. For high force cyclic loads, no difference was observed between meniscectomized and intact joints (P<0.05). At low force, a slight difference was present (82% ±4.7 vs 90% ±3.1, P<0.05). Due to the greater reduction in thickness, the cartilage thickness recovery was significantly prolonged following meniscectomy (Fig. 10).

The height of the acute bend in the collagen fibers at the center of indentation was significantly (P<0.05) closer to the subchondral bone in joints from which the meniscus had been removed. For example, after high force and long duration static loading, it was located at 48% (±6.1) of total fiber length from the fiber base in the intact cartilage, and at 20% (±8.3) after meniscectomy. At lower load, the location of bending was higher in the fiber, but a difference between intact and meniscectomized joints persisted (from 65% ±3.5 to 51%±4.8, P<0.05). Following meniscectomy the collagen fibers were crimped more tightly (in terms of wavelength) under static and cyclic loading compared to that in the intact joint (8 μm±0.5 vs 13 μm±1.1, for low force, long duration static loading) (Table I).

**ACL-TRANSECTION**

There were no significant differences in collagen fiber deformation under static load between ACL-transected and intact joints. Under cyclic loading, the level of collagen fiber bending was visibly deeper in the cartilage, when compared to the intact and meniscectomized joint [Fig. 6(b)]. Under cyclic loads, crimping was minimal or absent in the intact and meniscectomized joint, while after ACL-transection, the crimp was increased and localized in the region of the main bending angle [Fig. 6(b)].

After ACL-transection the area of tibial indentation and the minimum cartilage thickness were not different from that in the intact joint for static or cyclic loading situations (P>0.05) (Fig. 8). Following ACL-transection, the location of fiber bending was significantly lower (P<0.05) after high force cyclic loading (49.2%±4.6) compared to that in the intact (78.6%±3.6) and meniscectomized joints (72.6% ±3). ACL-transection was associated with more pronounced crimping under cyclic loads compared to that in the intact joint (period of 8 μm±1.3 vs 15 μm±0.7, for high force cyclic loading).

**Discussion**

In this study, articular cartilage collagen deformation in rabbit knee joints was examined following experimental lesions known to cause joint degeneration from clinical experience and animal experimental models. The findings revealed a significantly increased deformation of the tibial plateau and its articular cartilage collagen structure following meniscectomy under static loads but not under cyclic loads. The only changes detected after ACL-transection occurred under cyclic loading conditions, and these were less pronounced than those after meniscectomy. Some changes were more obvious with quantitative methods, but others were also obvious by qualitative morphological examination. Because this was an *in vitro* study performed immediately after the lesions were made, the results are interpreted as the acute consequences of isolated injuries.

The findings of this study are consistent with what is known about experimental meniscectomy and ACL-transection. Medial meniscectomy consistently induces
arthritic changes in the rabbit knee joint. Biomechanical studies in other species show that the menisci carry between 30% and 55% or more of the load across the knee joint. Removal of the meniscus increases the average stress across the knee joint by two to three times, presumably due to decreased surface contact. It is reasonable to assume that this increase in stress may lead to permanent damage to the matrix. Our observations are compatible with that theory of increased stress. The current study showed that the cartilage re-swells completely, indicating that the loading itself does not irreversibly damage the matrix. Our observations are compatible with that theory of increased stress. The current study showed that the cartilage re-swells completely, indicating that the loading itself does not irreversibly damage the matrix. Since this was an in vitro study, no damage of the matrix has been shown. Removal of the meniscus caused an increase of almost 80% in tibio-femoral indentation area and nearly 20% greater reduction of cartilage thickness under static loads.

The observation that meniscectomy did not increase deformation with high cyclic loads and produced only slightly increased deformation with low cyclic loads was unexpected. Perhaps joint motion moves the main area of contact, effectively distributing loads over a larger area on the plateau. The greater discrepancy (observed morphologically and quantitatively) between intact and meniscus-deficient joints at low cyclic loads can be explained by the fact that the meniscus normally bears a greater proportion of the total load when the load is small. Regardless of the mechanism, it is apparent that the joint movement reduces the extent of joint deformation at high loads. This finding could have relevance to the outcome of meniscectomy in humans, given the fact that mobilization following this surgery has varied over time.

In humans, the risk of OA following an isolated ACL rupture is still uncertain. Clearly, ACL deficiency leads to degeneration in canine knees and biomechanical/biochemical changes typical of early OA in the rabbit. In the study of Yoshioka et al., the changes associated with ACL-transection were less pronounced than with meniscal removal and were detected only in conditions where the joint was cyclically loaded. This suggests that tibial contact pressures indicated by collagen fiber deformation in the tibio-femoral contact area are not altered dramatically by transection of the rabbit ACL. On the other hand, Noyes found that ACL deficient subjects have higher joint reaction forces, probably created by the larger muscle forces required to stabilize the joint. However since our model did not simulate higher joint loads, our findings remain consistent with both studies. There is no obvious explanation for the fact that ACL-transection had the most
visible effects in knees that were moved, but this may be related to the instability of ACL-transected joints.\textsuperscript{48–50} Perhaps because of slight translation dictated by the ligaments, the overall contact surface between femur and tibia changes during the movement of intact joints.\textsuperscript{51} If this translation were reduced by ACL-transection, any resulting load concentration would lead to greater deformity. Our studies clearly show that total deformation is proportional to load duration as well as magnitude. More importantly, the smaller magnitude of acute cartilage deformation found following ACL section in comparison to that after meniscectomy seems to be consistent with the clinical observations of greater severity of OA following meniscectomy.

The techniques applied here to preserve cartilage in its natural state while under load are unique. Therefore, these SEM observations of the acute effects of loading on the three-dimensional collagen structure after meniscectomy or ACL transection are new. The preparation of articular cartilage samples for SEM has specific limitations which have been discussed previously.\textsuperscript{36,37,39} The joints are not totally frozen until 20 s following immersion in the pre-cooled isopentane. Ice crystal formation and drying-related shrinkage and cooling may cause artefactual separation of fibers. These effects are limited, and the collagen matrix structure following cryopreservation is remarkably similar to that in tissue fixed by conventional means but with significantly less shrinkage.\textsuperscript{39} In contrast to conventional methods, freeze-substitution fixation allows examination of entire articular surfaces preserved in a fully hydrated state loaded in non-equilibrium conditions. In contrast to jigs, which hold joints in a testing machine,\textsuperscript{21} our loading device places minimal constraint on tibial rotation and translation.
and permits joint flexion/extension when desired. We do not know the exact tibio-femoral joint reaction forces generated by the device, but the forces were applied in a standardized, reproducible manner to enable relative comparisons.

We observed a characteristic formation of clefts around the indentation, but fewer clefts in the indented area. We assume from the morphologic findings that fluid is forced out of the main region of compression and moves into regions with lower pressure. The clefts probably form during dehydration, caused by increased shrinkage in regions with higher water content. They are not present in the samples prior to dehydration. We believe that these clefts are preparation artefacts and may not be markers of cartilage micro-damage.36

Post-traumatic degenerative arthrosis is probably induced by a combination of mechanical abnormalities and cell-based reactions. Clearly, alteration of the collagenous matrix is present from the early stages following experimental meniscectomy, both at the light microscopic and ultrastructural levels.12,13,52–55 In human osteoarthritic cartilage, long-term effects upon the collagen structure, such as obliteration and separation of collagen fibers, fibrillation and decrease of fiber diameter, and cleft formation in the superficial and deep cartilage zones, are known.27,28,56,57

As Mankin31,32,58 and others34,59,60 state, it is reasonable to assume that much of this change reflects direct damage—which was not shown in the current study—to the matrix and represents an earlier stage of joint degeneration.

This study has documented patterns in which the collagen fibers are deformed in mechanically abnormal situations and has shown that these patterns are affected by loading condition; specifically load magnitude and joint movement. High stresses in the matrix may contribute to the initiation and progression of cartilage destruction following meniscectomy.61,62 The tissue strain indicated by collagen deformation observed with the larger total loads was remarkable. It is possible that increased crimping and acuteness of the bending angle increases shearing forces between the collagen fibers, and thus could lead to the early features of matrix degeneration, specifically, the sub-surface clefts and fibrillation. Proof of this, of course, is beyond the scope of the present study. Yet such information, gathered immediately after a specific injury, is adequate for visualizing the stresses and/or strains borne by the collagen matrix.

Conclusions

Alteration of knee joint biomechanics by meniscectomy or ACL-transection led to significant changes in the acute deformation of the cartilage collagen structure under mechanical load. Following meniscectomy and under static load, significantly increased deformation of cartilage collagen structure in the tibial plateau was found. After ACL-transection only cyclic loading resulted in a slightly increased deformation. Furthermore, the time which cartilage takes to completely recover is prolonged significantly after meniscectomy. These phenomena may be relevant for the initiation and progression of articular cartilage degeneration due to overload of the collagen structure.

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