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Site-dependent changes in structure and function of lapine articular cartilage 4 weeks after anterior cruciate ligament transection

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SUMMARY

Objective: The aim of this study was to investigate the site-dependent changes in the structure and function of articular cartilage in the lapine knee joint at a very early stage of osteoarthritis (OA), created experimentally by anterior cruciate ligament transection (ACLT).
Methods: Unilateral ACLT was performed in eight mature New Zealand white rabbits. ACL transected and contralateral (C-L) joints were prepared for analysis at 4 weeks after ACLT. Three rabbits with intact joints were used as a control group (CNTRL). Femoral groove, medial and lateral femoral condyles, and tibial plateaus were harvested and used in the analysis. Biomechanical tests, microscopy and spectroscopy were used to determine the biomechanical properties, composition and structure of the samples. A linear mixed model was chosen for statistical comparisons between the groups.
Results: As a result of ACLT, the equilibrium and dynamic moduli were decreased primarily in the femoral condyle cartilage. Up to three times lower moduli ($P < 0.05$) were observed in the ACLT group compared to the control group. Significant ($P < 0.05$) proteoglycan (PG) loss in the ACLT joint cartilage was observed up to a depth of 20–30% from the cartilage surface in femoral condyles, while significant PG loss was confined to more superficial regions in tibial plateaus and femoral groove. The collagen orientation angle was increased ($P < 0.05$) up to a cartilage depth of 60% by ACLT in the lateral femoral condyle, while smaller effects, but still significant, were observed at other locations. The collagen content was increased ($P < 0.05$) in the middle and deep zones of the ACLT group compared to the control group samples, especially in the lateral femoral condyle.
Conclusion: Femoral condyle cartilage experienced the greatest structural and mechanical alterations in very early OA, as produced by ACLT. Degenerative alterations were observed especially in the superficial collagen fiber organization and PG content, while the collagen content was increased in the deep tissue of femoral condyle cartilage. The current findings provide novel information of the early stages of OA in different locations of the knee joint.

Introduction

Anterior cruciate ligament transection (ACLT) (the Pond-Nuki model[1]) is a recognized method to induce osteoarthritis (OA) in animal models[2–7]. Without the ACL resisting primary anterior tibial displacement and internal tibial rotation, loading patterns of the knee joint change[8], leading to degenerative changes in metabolism and structure of articular cartilage[9]. These changes are characteristic markers of early OA[10]. ACL rupture can lead to OA even with reconstructive surgery[11], but if the very first mechanisms responsible for the tissue changes are known, strategies may be developed to stop or limit the progression of OA.

Cartilage consists mainly of interstitial water, collagen (type II) fibers, and proteoglycans (PGs)[12]. The fluid phase (water) primarily

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resists instantaneous loads and transient, poroelastic responses of the tissue, while collagen fibers resist mainly tensile stresses. Under prolonged load, when fluid flow in the tissue has ceased, the PG mesh resists cartilage compression[12]. The first degenerative changes of cartilage structure have been observed to start from the superficial layer where PG content decreases and collagen fibrillation occurs[6,7,13–18]. When cartilage also swells, increased fluid content and reduced solid content in the degenerated cartilage allows for easier flow of the interstitial water out of the loaded cartilage. These structural changes lead to alterations in the biomechanical properties of articular cartilage; permeability increases and dynamic and equilibrium stiffness decrease[7,19].

In, *in vivo* experimental studies aimed at identifying the first signs of OA, different animals (e.g., dogs[14,20], rabbits[3,13,21–23], rodents[6], sheep[24]) and mechanical interventions (e.g., ligament transection[13,23], meniscectomy[22,25], traumatic impact[26], combination of these[20,27,28]) have been used. Simultaneous transection of the anterior and posterior cruciate ligaments combined with meniscectomy have been shown to lead to noticeable degradation of articular cartilage as early as 2 weeks after the intervention[29]. However, in ACL transected rabbits, the first signs of cartilage deterioration have been reported to occur at 4 weeks[3,13,21]. Superficial PG loss and degradation of the collagen matrix organization have been detected, while no significant changes in the collagen content have been observed.

Earlier studies investigating the effect of ACLT on degenerative changes of cartilage have usually concentrated on either the femoral, the tibial or the patellar cartilage[3,13,20,21,23,27]. Results of structural and mechanical differences between sites in the knee, especially with a depth-wise structural analysis and a very short time period after transection, are lacking. Also in ACLT models, unilateral transection is used as the golden standard, with the contralateral side often used as the control[5,6,13,14,30,31]. However, evidence of systematic changes in contralateral limbs exist[23], thus the question arises whether the contralateral joint should be used as an unaffected control.

The aim of this study was to investigate the structural and functional changes of articular cartilage in the lapine knee, and the site-dependence of these changes at a very early stage of OA, created experimentally by ACLT. Biomechanical measurements were used to analyze the changes in the elastic properties of cartilage. Microscopic and spectroscopic methods were applied to analyze depth-dependent composition, i.e., collagen and PG content and collagen orientation of the samples. The femoral groove, lateral and medial femoral condyles, and tibial plateaus of transected, contralateral and non-transected control knees were used for site-specific analysis. To our knowledge, this is the first time these early changes in lapine articular cartilage structure and function have been investigated in a site-specific manner, only at 4 weeks after ACLT.

Methods

A brief presentation of the materials and methods is given here. More details are presented in the Supplementary Material.

Animal model

Skeletally mature, female New Zealand white rabbits (Oryctolagus cuniculus, age 14 months, $n = 11$) were used for all tests. Unilateral ACLT was performed in eight rabbits and the contralateral joints were used for analysis as a contralateral (C-L) group[13]. Six knee joints from three non-operated rabbits were used as a separate control group (CNTRL). Animals were sacrificed at 4 weeks following ACLT and knee joints were dissected and immersed into phosphate buffered saline (PBS). ACLT and C-L group knees were frozen in dry ice and shipped from Calgary, Alberta, Canada to Kuopio, Finland. The CNTRL group samples were tested biomechanically first in Calgary before shipping them to Kuopio in formalin fixative. ACLT procedures for rabbits were approved by the Animal Ethics committee at the University of Calgary and the guidelines of the Canadian Council on Animal Care were followed.

Biomechanical tests

For biomechanical measurements, cartilage-on-bone samples were harvested [Fig. 1]. Biomechanical measurement locations were defined as the top point (apex of the posterior curvature) of femoral condyles and groove and the central point of tibial plateaus for femur and tibia, respectively[32,33]. Samples were glued to the bottom of a measuring chamber for each measurement and the chamber was filled with PBS. A stress-relaxation protocol was implemented using a ramp rate of 100 %/s (350 ± 150 μm/s), 3 × 5% steps and 15 min relaxation time after each step, after which a sinusoidal dynamic test was performed with a frequency of 1 Hz (amplitude 4% of remaining tissue thickness, four cycles). Both equilibrium and dynamic linearly elastic moduli were then determined similarly as earlier[34–36].

Microscopic and spectroscopic analysis

After biomechanical measurements, the samples were fixed in formalin, decalcified, processed in graded alcohol solutions and embedded in paraffin. Histological sections were then cut perpendicular to the cartilage surface. Digital densitometry (DD) sections (thickness ~3 μm) were stained with Safranin O[37] while

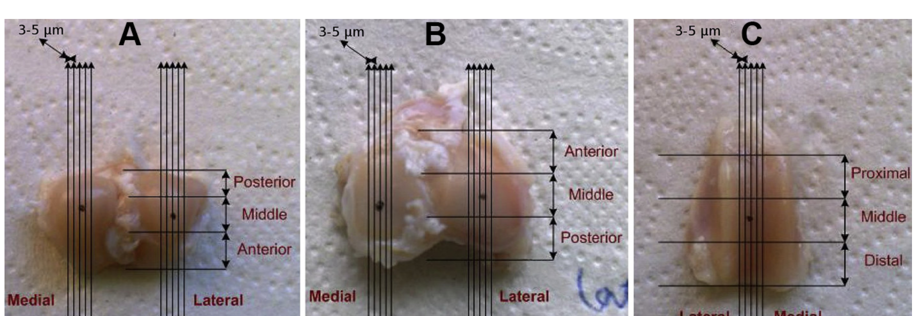

Fig. 1. Analyzed locations in the lapine knee joints. Articular cartilage samples in the lateral and medial femoral condyles (A), tibial plateaus (B) and femoral groove (C) were biomechanically tested and used for determinations of tissue structure and composition. Indentation locations are marked with black dots, while the directions of slices prepared for microscopy and spectroscopy are marked with solid lines. Spaces between lines are not to scale.

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