Osteoarthritis and Cartilage 23 (2015) 414–422

Osteoarthritis and Cartilage

Alterations in subchondral bone plate, trabecular bone and articular cartilage properties of rabbit femoral condyles at 4 weeks after anterior cruciate ligament transection

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ARTICLE INFO

Article history:
Received 13 August 2014
Accepted 25 November 2014

Keywords:
Subchondral bone plate
Trabecular bone
Osteoarthritis
Anterior cruciate ligament transection
Micro-computed tomography
Articular cartilage

SUMMARY

Objective: To quantify early osteoarthritic-like changes in the structure and volume of subchondral bone plate and trabecular bone and properties of articular cartilage in a rabbit model of osteoarthritis (OA) induced by anterior cruciate ligament transection (ACLT).

Methods: Left knee joints from eight skeletally mature New Zealand white rabbits underwent ACLT surgery, while the contralateral (CTRL) right knee joints were left unoperated. Femoral condyles were harvested 4 weeks after ACLT. Micro-computed tomography imaging was applied to evaluate the structural properties of subchondral bone plate and trabecular bone. Additionally, biomechanical properties, structure and composition of articular cartilage were assessed.

Results: As a result of ACLT, significant thinning of the subchondral bone plate ($P < 0.05$) was accompanied by significantly reduced trabecular bone volume fraction and trabecular thickness in the medial femoral condyle compartment ($P < 0.05$), while no changes were observed in the lateral compartment. In both lateral and medial femoral condyles, the equilibrium modulus and superficial zone proteoglycan (PG) content were significantly lower in ACLT than CTRL joint cartilage ($P < 0.05$). Significant alterations in the collagen orientation angle extended substantially deeper into cartilage from the ACLT joints in the lateral femoral condyle relative to the medial condyle compartment ($P < 0.05$).

Conclusions: In this model of early OA, significant changes in volume and microstructure of subchondral bone plate and trabecular bone were detected only in the femoral medial condyle, while alterations in articular cartilage properties were more severe in the lateral compartment. The former finding may be associated with reduced joint loading in the medial compartment due to ACLT, while the latter finding reflects early osteoarthritic changes in the lateral compartment.

Introduction

Osteoarthritis (OA) is a complex degenerative disease affecting not only articular cartilage, but also the entire joint including synovium, menisci, ligaments and subchondral bone[1]. In advanced stages, OA is primarily associated with articular cartilage degeneration and subchondral bone sclerosis (increased bone density and volume). Radin *et al.*[2] were the first to suggest the potential role for subchondral bone in the initiation and progression of OA. They stated that alterations in the subchondral bone stiffness increase

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http://dx.doi.org/10.1016/j.joca.2014.11.023
1063-4584/

stresses in the overlying articular cartilage and eventually lead to tissue degeneration.

When considering the contribution of bone in OA, it is important to distinguish between two structures, particularly the subchondral bone plate and the trabecular bone, as they are different in morphology and mechanical properties. Their responses are also different during OA progression[3]. Subchondral bone plate thickening[4] and an increase in the trabecular bone volume fraction[5] have been reported in patients with late stage of OA. Additionally, it has been suggested that these subchondral bone changes are related to the severity of the cartilage lesions[6]. Nevertheless, whether changes in the subchondral bone plate and/or trabecular bone accompany, precede or follow cartilage degeneration at the onset and very early stages of the disease are still unclear. This limited information on OA is partly due to the absence of valuable means to detect early changes or to follow the natural history of the disease in human patients.

Surgically-induced animal models of OA, such as an anterior cruciate ligament transection (ACLT) (the Pond–Nuki model[7]) generally involve inducing a mechanical instability within a joint of interest, leading to pathological changes similar to those observed in post-traumatic human OA[8–11]. Structural, compositional and mechanical changes have been shown to occur in cartilage using various ACLT animal models[12–15]. In canine ACLT models, subchondral bone loss has been reported to occur in early OA[16,17], followed by bone sclerosis at later stages[18]. In an ACLT feline model, however, a long-term thinning of the subchondral plate was observed[19]. The differences seen in these surgically-induced OA models cannot only be explained by the use of different animals, but also by the different post-surgical time points used for monitoring the changes.

The rabbit ACLT model is increasingly being used in early OA studies, as it is known to induce rapid and severe changes in articular cartilage[9,20,21] and subchondral bone[21–23]. Recent evidence indicates that early osteoarthritic changes in the structure and composition of rabbit articular cartilage following ACLT occur in a depth-dependent manner[24,25] and are highly site-specific[26] with most severe changes occurring in the femoral condylar cartilage. However, only a few studies[21] have characterized early changes in both articular cartilage and subchondral bone in the rabbit ACLT model. For instance, Batiste *et al.*[21] found decreased compartmental bone mineral density (BMD) levels and changes in macroscopic surface integrity of rabbit articular cartilage as early as 4 weeks post-surgery. To our knowledge, none of the previous studies characterized early osteoarthritic changes in the subchondral bone plate and trabecular bone with depth-wise changes in cartilage structure and composition in the rabbit ACLT model.

The aim of the present study was to quantitatively determine the changes in the properties of subchondral bone plate, trabecular bone and articular cartilage in rabbit femoral condyles as early as 4 weeks post-ACLT. A high-resolution microCT scanner was used to assess the three-dimensional microarchitecture and volume in the subchondral bone plate and trabecular bone. Additionally, depth-wise changes in the proteoglycan (PG) content, collagen orientation angle and collagen content and as well biomechanical properties of cartilage were quantified using digital densitometry (DD), polarized light microscopy (PLM), Fourier Transform Infrared (FTIR) microspectroscopy and indentation testing, respectively.

Methods

Animals and procedure

Skeletally mature female New Zealand white rabbits (*Oryctolagus cuniculus*, $n = 8$, age 14 months, weight 5.4 ± 0.6 kg) underwent unilateral ACLT under general anesthesia. The left knee was operated, while the right knee (contralateral joint) was used as a non-transected control group. Femoral condyles were harvested 4 weeks after the ACLT. All procedures were approved by the Committee on Animal Ethics at the University of Calgary and were carried out according to the guidelines of the Canadian Council on Animal Care. These animals were originally processed for our earlier studies[24,26], in which only the properties of cartilage were analyzed. For the present study, femoral condyle bones were imaged and analyzed, while femoral cartilage properties were implemented for comparison.

Micro-computed tomography

After biomechanical measurements of cartilage (see the subsection on the *Mechanical, structural and compositional analysis of articular cartilage* below), distal compartments of femoral condyles were imaged using a high-resolution cone-beam microCT scanner (Skyscan 1172, Aartselaar, Belgium) with an isotropic voxel size of 25 μm and a 0.5 mm thick aluminum filter. The X-ray tube voltage was 100 kV and the current was 100 μA. The X-ray projections were obtained at 0.7° rotation step with 316 ms exposure time. The cross-sectional images were reconstructed using a modified Feldkamp cone-beam algorithm (NRecon software, v.1.6.2.0, Skyscan, Aartselaar, Belgium). The reconstructed microCT data was first imported into Mimics software (v.12.3, Materialise, Belgium) for visualizing the 3D geometry of femoral condyles and segmentation. To distinguish bone tissue from other tissues, all reconstructed grayscale images were segmented, using a fixed global threshold defined by visual inspection of the segmentation result. Subsequently, $2 \times 2 \times 4$ mm^3 volumes of interest (VOIs) were placed in weight-bearing regions of medial and lateral femoral condyles [Fig. 1]. Similarly as was done before[26,27], the weight-bearing regions were selected at the apex of each femoral condyle. Using Mimics software, the VOIs containing both subchondral bone plate and trabecular bone were further manually segmented using a contour-based tool in order to delineate the two components [Fig. 1]. The subchondral bone plate and trabecular bone were segmented manually a few voxels away from the endocortical boundary using previously described criterion based on the size of intracortical pores[28]. According to this criterion[28], the endocortical boundary splits the pore in the case of large pores, whereas the pore was included in the subchondral plate region if the size of a pore was less than twice the average size of pores in that region or if the pore size was smaller than the distance from the pore to the endosteal region (i.e., the border between the bone and the bone marrow). Repeatability for the manual segmentation was high with intraclass correlation coefficient (ICC) values of 0.94 for the medial subchondral bone plate thickness (95% confidence intervals (CI), 0.83–0.98) and 0.95 for the lateral subchondral bone plate thickness (95% CI, 0.85–0.98). This test estimated the correlation between three repeated measurements for each sample from the contralateral (CTRL) group ($n = 8$).

After separation, the segmented masks containing the subchondral bone plate and trabecular bone were independently processed and further imported into CTAn software (Skyscan, v.1.13, Aartselaar, Belgium), using a custom made Matlab script (The MathWorks, Inc., MA, v.7.14.0). Image processing included Gaussian (sigma = 0.8, support = 1) and despeckle filtering (3D, white speckles of <8 voxels were removed). Subchondral bone plate and trabecular bone structural parameters were calculated with CTAn software according to American Society for Bone and Mineral Research (ASBMR) guidelines and computed in a direct 3D fashion based on the marching cubes algorithm without any model assumptions for 2D analysis[29]. The calculated 3D morphometric