Early changes in osteochondral tissues in a rabbit model of post-traumatic osteoarthritis

Lingwei Huang¹ | Ilari Riihioja² | Petri Tanska¹ | Simo Ojanen¹,³ | Sanna Palosaari²,⁴ | Heikki Kröger⁵ | Simo J. Saarakkala³,⁶ | Walter Herzog⁷,⁸ | Rami K. Korhonen¹ | Mikko A. J. Finnilä¹,³,⁶

¹Department of Applied Physics, University of Eastern Finland, Kuopio, Finland
²Medical Research Center, Bone and Stem Cell Biology Research Group, University of Oulu and Oulu University Hospital, Oulu, Finland
³Research Unit of Medical Imaging, Physics and Technology, Faculty of Medicine, University of Oulu, Oulu, Finland
⁴Cancer and Translational Medicine Research Unit, Anatomy and Cell Biology, Faculty of Medicine, University of Oulu, Oulu, Finland
⁵Department of Orthopedics, Traumatology and Hand Surgery, Kuopio University Hospital, Kuopio, Finland
⁶Department of Diagnostic Radiology, Oulu University Hospital, Oulu, Finland
⁷Human performance laboratory, Faculty of Kinesiology, University of Calgary, Calgary, Alberta, Canada
⁸Biomechanics Laboratory, School of Sports, Federal University of Santa Catarina, Florianópolis, Santa Catarina, Brazil

Correspondence
Lingwei Huang, Yliopistonranta 1 F, 70210 Kuopio, Finland.
Email lingwei.huang@uef.fi and huang_lingwei@163.com

Abstract
Concurrent osteoarthritic (OA) manifestations in bone and cartilage are poorly known. To shed light on this issue, this study aims to investigate changes in subchondral bone and articular cartilage at two time points after anterior cruciate ligament transection (ACLT) in a rabbit model. 2 (N = 16) and 8 (N = 10) weeks after ACLT, the subchondral bone structure, cartilage thickness, Osteoarthritis Research Society International (OARSI) score, fixed charged density (FCD), and collagen orientation angle were analyzed. OA related changes were evaluated by comparing the ACLT to the contralateral (C-L) and control knees. Already 2 weeks after ACLT, higher trabecular number in the medial femoral condyle and femoral groove, greater OARSI score in the femoral condyles, and thinner trabeculae in the lateral tibial plateau and femoral groove were observed in ACLT compared to C-L knees. Only minor changes of cartilage collagen orientation in the femoral condyles, femoral groove and patella were observed. 8 weeks post-ACLT, the surgical knees had thinner subchondral plate and trabeculae, and smaller trabecular bone volume fraction in most of the knee locations. OARSI score was greater in the femoral condyle and lateral tibial plateau cartilage. FCD loss was progressive only in the femoral condyle, femoral groove, and patellar cartilage, and minor changes of cartilage collagen orientation angle were present in the femoral condyles, femoral
1 | INTRODUCTION

Post-traumatic osteoarthritis (PTOA) is a clinical phenotype of OA caused by joint trauma. Substantial ligament or capsule injuries, or articular fractures, increase the risk of PTOA. Anterior cruciate ligament transection (ACLT) is a commonly used method for inducing PTOA in animal models. ACLT leads to progressive cartilage degeneration, as characterized by an increase in chondrocyte apoptosis, proteoglycan (PG) depletion, roughening of the articular surfaces initially, and cartilage delamination and excavation later in the disease process. These structural changes lead to decreased intrinsic moduli and increased hydration and hydraulic permeability of articular cartilage. Cartilage fibrillation has been reported in the medial femoral condyle 2 weeks after ACLT, followed by osteophyte formation, cartilage erosion, and full thickness ulceration 8 weeks post-ACLT in the femoral condyles of rabbit knees. 4 weeks after a unilateral ACLT, the number of apoptotic chondrocytes in the superficial zone of the cartilage increased, and cell apoptosis appeared in the middle zone. In addition, ACLT can also induce changes in the composition and collagen organization of articular cartilage in rabbit knees. 9 weeks after ACLT, the glycosaminoglycan content decreased by 11%, and the water content increased by 7% in articular cartilage from the medial femoral condyle. Similarly, the collagen network was unchanged and the PG content was reduced in the superficial zone of articular cartilage 9 weeks post-ACLT, and significant alterations in the collagen network were extended deeper in the lateral than the medial femoral condyle.

Subchondral bone is a mechanosensitive tissue providing mechanical support for articular cartilage. Structural changes in the subchondral bone have been thought to occur prior to any observable degeneration in articular cartilage. It is still unclear how the cartilage and subchondral bone change concurrently in the onset and progression of early OA. In primary (idiopathic) OA, a positive association between the Osteoarthritis Research Society International (OARSI) grade and the subchondral bone plate thickness was discovered, and aberrant bone formation was observed in the proximal tibias of total knee arthroplasty patients. Studies on the human tibial plateaus from OA patients and the medial condyles from guinea pigs with spontaneous OA showed a reduction in trabecular rods and an increase in trabecular thickness. During OA progression, the bone turnover, thinning of the trabecular structure, osteophytes, bone marrow lesions, and sclerosis of the subchondral bone increased. There have been also various studies on animal models of PTOA. These have reported bone loss in the femoral condyles and tibial plateaus 4 and 8 weeks after ACLT surgery, accompanied by a reduced bone mineral density.

Based on the previous observations, research on timing of structural changes of bone and cartilage after ACLT in rabbits is lacking. In PTOA rabbit models, cartilage fibrillation, minor collagen orientation changes and significant fixed charged density (FCD) loss were observed 2 weeks after ACLT, till full thickness cartilage ulceration 8 weeks post-ACLT. The ACLT-induced morphological degeneration of articular cartilage and subchondral bone was retained the same from 8 to 16 weeks. Thus, 2 weeks post-ACLT seemed an appropriate earliest time point to study the onset and very early changes of PTOA, and 8 weeks post-ACLT should provide good information about the progress of these earliest OA changes. Furthermore, most previous studies focused on changes in either articular cartilage or subchondral bone, or were limited to a few locations and one time point, and therefore, they could not provide a comprehensive site-specific understanding of the initiation and progression of PTOA.

The aim of this study was to characterize the concurrent changes in subchondral morphology, as well as the collagen orientation and PG content of articular cartilage in PTOA rabbit knee joints 2 (early OA) and 8 (advanced OA) weeks after ACLT. Since thickness of cartilage and loading mode change in respect of anatomical region of knee, we hypothesized that ACLT induced changes are site-specific and have time-dependent progression. We further hypothesized that changes to the bones occur first in the tibia under thick cartilage, while changes in articular cartilage occur first in the femur with thin cartilage.

2 | METHODS

2.1 | Rabbit model and sample preparation

This study was approved by the committee on Animal Ethics at the University of Calgary (Renewal 3 for ACC Study #AC110035), and all experiments were conducted under the guidelines of the Canadian Council on Animal Care. Skeletally mature female New Zealand White rabbits (Oryctolagus cuniculus, 12 ± 0.5 months, 4.98 ± 0.45 kg) were used in the experiments. All the animals were housed in single cages and unilateral ACLT surgery was conducted on a random knee of 14 random rabbits. The main procedure of the sample processing is shown in Figure 1. Animals were sacrificed 2 or 8 weeks after the ACLT surgery.
The detailed procedure of anesthesia, operative approach, postoperative analgesia, and euthanasia have been described previously.23 Shortly, all the rabbits in the surgery group were injected subcutaneously with painkillers twice daily for 2 days. At the appropriate time points, rabbits were sacrificed by intracardiac injection of pentobarbital sodium for the collection of knee samples. Knees were collected as controls. Considering potential systematic effects, one additional group (CNTRL) of eight control samples were collected from non-operated age-matched rabbits (N = 8 rabbits, at 2-week time point; N = 4 rabbits, at 8-week time point). After careful dissection of the joints, osteochondral samples were prepared from six anatomical locations: (i) lateral and (ii) medial femoral condyles and (iii and iv) tibial plateaus, (v) femoral groove and (vi) patella.

### 2.2 Subchondral structure evaluation using micro-computed tomography

After fixation with formalin, samples were wrapped in moist papers and placed in plastic vials for micro-computed tomography (µCT) imaging (Skyscan 1272; Bruker microCT). The X-ray tube for the µCT was set to 50 kV, and a 0.5-mm-thick aluminum filter was applied. 270 projection images were collected every 0.7° using 1450 s integration time and frame averaging of 2. Images with a pixel size of 25 µm were reconstructed in NRecon software where beam hardening corrections were applied. For further details, please see the Supplementary Material. One volume of interest (VOI) with a size of 80 × 80 pixels × sample height was selected from the central load bearing area of each target location (Figure S1). To generate VOI_trab, the trabecular bone was manually segmented with an analysis software (CTAn, Version 1.16.4.1), as described previously,12 and the subchondral plate VOI_plate was acquired with a shrink-wrap operation on images where trabecular bone had been removed (VOI - VOI_trab). Three-dimensional (3D) morphometric analyses of the subchondral plate and trabecular bone were conducted separately, and the subchondral plate thickness (cross-sectional thickness from two-dimensional analysis), trabecular bone volume fraction, trabecular thickness (based on Euclidian distance transform), trabecular number, and trabecular separation were assessed.

### 2.3 Articular cartilage assessment

Before µCT imaging, the thickness of articular cartilage was measured by using optical coherence tomography (Illumien PCI Optimization System; Jt. Jude Medical) as previously described.24 After the µCT imaging, samples were processed for histology with ethylenediaminetetraacetic acid decalcification, followed by dehydration and paraffin embedding. Three 3-µm-thick sections were cut from the central load bearing area of each location of the rabbit knee joints and then these sections were stained with Safranin-O, and three 5-µm-thick sections were cut and left unstained. Microscopic images of the Safranin-O stained sections were collected with a digital pathology slide scanner (Aperio AT2; Leica Biosystems) using 40 × magnification. OARSI score was assessed from these images according to the established histopathology standards by the OARSI25 (Figure 2 and Supplementary Material). The three Safranin-O stained sections were also imaged with digital densitometry26,27 for analyzing FCD content based on a positive and linear relationship...
between FCD and optical density \(^{27,28}\) (see Supplementary Material). To avoid artefacts in the structural analysis caused by non-homogenous staining, unstained sections were used for the assessment of collagen orientation.\(^{29}\) The depth-wise collagen orientation angle (0°-parallel to cartilage surface, 90°-perpendicular to cartilage surface) was evaluated from unstained sections with a quantitative polarized light microscope\(^ {30}\) (Supplementary Material). The distribution profiles of the collagen orientation angle and FCD were laterally averaged for each section, and then the averaged profiles were averaged again over these three sections. The finally acquired averaged profiles were analyzed from a 150-µm-wide region (from the cartilage surface to the cartilage-bone interface) to form the depth-wise collagen orientation angle and FCD profiles.

### 2.4 Statistical analysis

For the depth-wise statistical comparisons of cartilage structure, the FCD and collagen orientation profiles were linearly interpolated to 100 points. A linear mixed regression model without confidence interval adjustment was used for testing differences in the bone morphology, cartilage thickness, OARSI score, as well as the depth-wise collagen orientation angle and FCD between the ACLT, C-L and CNTRL groups 2 or 8 weeks after surgery. In addition to the analysis of depth-wise collagen orientation angle and FCD, the averaged collagen orientation angle and averaged FCD in the superficial layer of articular cartilage (within 0%–20% of cartilage depth) were also analyzed. In the model, animal identification and knee side (left/right) were defined as random effects, and the experimental groups (i.e., the ACLT, C-L, and CNTRL groups) as fixed effects. All the results are presented in the format of mean ± standard deviation and the significance level for all tests was \(\alpha = 0.05\).

### 3 RESULTS

#### 3.1 The assessment of subchondral structure deterioration

Representative 3D visualization of the subchondral plate and trabecular bone in each group are shown in Figure 3. 2 weeks after the ACLT

---

**FIGURE 2** Visualization of cut histology images (Safranin-O staining) of osteochondral structure from the lateral and medial femoral condyles and tibial plateaus, femoral groove and patella of rabbit knees in the CNTRL, C-L, and ACLT groups 2 and 8 weeks after the surgery. The articular cartilage degenerates in the femoral condyles of the 2-week ACLT group against the C-L group and degenerates in the femoral condyles and lateral tibial plateau of the 8-week ACLT group against the C-L group. The degeneration of articular cartilage in the medial tibial plateau of the CNTRL and C-L group is much more than that of the ACLT group. The size of shown images: 2 mm \(\times\) height. The images before cutting were used for the OARSI score evaluation. ACLT, anterior cruciate ligament transection; C-L, contralateral; CNTRL, age-matched control; OARSI, Osteoarthritis Research Society International [Color figure can be viewed at wileyonlinelibrary.com]
surgery, a significantly higher trabecular number in the medial femoral condyle (2.14%, $p = 0.003$) and femoral groove (9.09%, $p = 0.019$), and lower trabecular thickness in the lateral tibial plateau (−6.67%, $p = 0.007$) and the femoral groove (−10.91%, $p = 0.015$) were discovered in the ACLT group, compared to the C-L group (Table 1). There were no significant differences in the subchondral bone structure between the ACLT and CNTRL groups 2 weeks after ACLT. Compared to the CNTRL group, thinner trabeculae in the patella (−44.64%, $p = 0.016$) and lower trabecular number in the femoral groove (−10.93%, $p = 0.038$) were found in the C-L group.

8 weeks after the ACLT surgery, the subchondral plate was thinner in the lateral tibial plateau (−26.19%, $p = 0.022$), femoral groove (−19.77%, $p = 0.005$) and patella (−19.52%, $p = 0.001$) in the ACLT group when compared to the C-L group (Table 2). In addition, the trabecular bone volume fraction was smaller in the 8-week ACLT group than the C-L group in the lateral (−13.76%, $p = 0.025$) and medial (−14.05%, $p = 0.013$) femoral condyles, lateral tibial plateau (−26.67%, $p < 0.001$) and femoral groove (−31.64%, $p < 0.001$). The trabeculae were thinner in the lateral (−11.10%, $p = 0.049$) and medial (−14.71%, $p = 0.003$) femoral condyles, lateral (−31.17%, $p = 0.004$) and medial (−16.53%, $p = 0.007$) tibial plateaus, femoral groove (−22.25%, $p = 0.004$) and patella (−7.31%, $p = 0.034$). Comparing the 8-week ACLT group to the CNTRL group, the trabecular bone volume fraction and trabecular thickness were smaller in the lateral (−14.35%, $p = 0.013$; −8.88%, $p = 0.041$) and medial (−11.30%, $p = 0.036$; −8.67%, $p = 0.049$) femoral condyles, lateral (−25.20%, $p < 0.001$; −25.33%, $p = 0.01$) and medial (−20.77%, $p = 0.002$; −16.13%, $p = 0.002$) tibial plateaus, with smaller trabecular bone volume fraction in the femoral groove (−14.35%, $p = 0.001$), and reduced subchondral plate thickness in the patella (−23.31%, $p < 0.001$), and greater trabecular separation in the medial tibial plateau (30.97%, $p = 0.015$) and femoral groove (33.40%, $p = 0.038$). When comparing the 8-week C-L group to the CNTRL group, we found significantly greater trabecular separation in the medial tibial plateau (32.33%, $p = 0.011$) and thicker subchondral plates in the femoral groove (15.46%, $p = 0.031$).

### Figure 3
3D µCT visualization of subchondral structure (including subchondral plate (red) and trabecular bone (blue)) from the lateral and medial femoral condyles and tibial plateaus, femoral groove and patella of rabbit knees in the CNTRL, C-L, and ACLT groups 2 and 8 weeks after the surgery. There are no differences in subchondral plate thickness between the CNTRL, C-L, and ACLT groups at the 2-week time point and the subchondral plate was thinner in the lateral tibial plateau, femoral groove and patella of the 8-week ACLT group against the C-L group. Trabecular bone is thinner in the lateral tibial plateau and femoral groove of the 2-week ACLT group against the C-L group, and is thinner in all the locations of the 8-week ACLT group against the C-L group. The size of volumes of interest: 2 × 2 mm × height. ACLT, anterior cruciate ligament transection; C-L, contralateral; CNTRL, age-matched control; µCT, micro-computed tomography [Color figure can be viewed at wileyonlinelibrary.com]
**TABLE 1** Subchondral bone structure in different anatomical locations of rabbit knees 2 weeks after the surgery

| Anatomical location in rabbit knees | Subchondral plate thickness (µm) | Trabecular bone volume fraction (%) | Trabecular thickness (µm) | Trabecular separation (µm) | Trabecular number (mm⁻¹) |
|------------------------------------|----------------------------------|------------------------------------|---------------------------|---------------------------|-------------------------|
|                                    | ACLT    | C-L     | CNTRL   | ACLT    | C-L     | CNTRL   | ACLT    | C-L     | CNTRL   | ACLT    | C-L     | CNTRL   |
| Lateral femoral condyle            | 443.3 ± 69.8 | 424.6 ± 51.7 | 463.0 ± 71.5 | 41.1 ± 2.2 | 43.3 ± 1.0 | 425 ± 5.6 | 195.3 ± 19.7 | 201.3 ± 12.1 | 188.1 ± 15.5 | 344.0 ± 38.2 | 325.3 ± 26.0 | 329.3 ± 41.5 | 2.13 ± 0.26 | 2.15 ± 0.10 | 2.26 ± 0.25 |
| Medial femoral condyle             | 426.9 ± 35.1 | 424.1 ± 30.9 | 449.6 ± 59.8 | 46.7 ± 4.0 | 46.8 ± 3.7 | 45.5 ± 4.9 | 196.1 ± 18.1 | 200.2 ± 9.4 | 195.0 ± 13.3 | 295.4 ± 45.2 | 295.2 ± 39.5 | 307.5 ± 35.1 | 2.39 ± 0.21 | 2.34 ± 0.19 | 2.33 ± 0.18 |
| Lateral tibial plateau              | 478.8 ± 62.3 | 442.9 ± 46.9 | 480.0 ± 69.2 | 47.7 ± 4.8 | 48.3 ± 8.8 | 49.8 ± 5.0 | 214.1 ± 21.1 | 229.4 ± 20.5 | 219.7 ± 57.5 | 317.9 ± 57.5 | 322.0 ± 31.0 | 311.0 ± 57.5 | 2.26 ± 0.37 | 2.10 ± 0.28 | 2.28 ± 0.24 |
| Medial tibial plateau               | 457.2 ± 60.7 | 429.1 ± 18.4 | 443.3 ± 37.3 | 43.3 ± 4.0 | 40.9 ± 6.0 | 45.3 ± 7.4 | 194.8 ± 33.9 | 224.4 ± 61.8 | 227.3 ± 33.9 | 325.4 ± 143.0 | 409.0 ± 86.7 | 388.7 ± 92.6 | 2.74 ± 0.53 | 1.83 ± 0.24 | 2.04 ± 0.53 |
| Femoral groove                     | 394.9 ± 34.8 | 397.7 ± 85.7 | 417.5 ± 46.7 | 37.3 ± 4.4 | 38.6 ± 4.1 | 41.0 ± 5.6 | 156.0 ± 15.3 | 175.1 ± 16.0 | 165.9 ± 9.9 | 306.1 ± 43.0 | 307.2 ± 22.3 | 289.9 ± 42.9 | 2.40 ± 0.27 | 2.20 ± 0.16 | 2.47 ± 0.28 |
| Patella                            | 637.6 ± 44.9 | 621.4 ± 75.5 | 683.4 ± 90.1 | 71.7 ± 8.2 | 66.2 ± 6.5 | 73.6 ± 6.8 | 290.3 ± 135.1 | 197.3 ± 101.1 | 356.4 ± 105.8 | 203.3 ± 68.7 | 171.4 ± 85.1 | 222.1 ± 47.0 | 3.89 ± 4.57 | 5.62 ± 5.29 | 2.24 ± 0.76 |

Note: *Significant difference to the CNTRL group, *p < 0.05, **p < 0.01, ***p < 0.001; mean ± SD. *Significant difference between the ACLT and C-L group, *p < 0.05, **p < 0.01, ***p < 0.001; mean ± SD. Abbreviations: ACLT, anterior cruciate ligament transection; C-L, contralateral; CNTRL, age-matched control.
| Anatomical location in rabbit knees | Subchondral bone structure (µm) | Trabecular bone volume fraction (%) | Trabecular thickness (µm) | Trabecular separation (µm) | Trabecular number (mm⁻¹) |
|-----------------------------------|--------------------------------|---------------------------------|--------------------------|---------------------------|-------------------------|
|                                   | Subchondral plate thickness | Trabecular bone volume          | Trabecular thickness     | Trabecular separation     | Trabecular number       |
|                                   | (µm)                        | fraction (%)                    | (µm)                     | (µm)                      | (mm⁻¹)                  |
| Lateral femoral condyle           | 381.2 ± 88.3                | 37.6 ± 3.3*                     | 168.2 ± 14.0*            | 339.7 ± 54.7              | 2.24 ± 0.22             |
|                                   | 416.2 ± 58.4                | 43.6 ± 4.9                      | 189.2 ± 9.1              | 313.9 ± 50.1              | 2.30 ± 0.21             |
|                                   | 354.3 ± 69.6                | 43.9 ± 4.2                      | 184.6 ± 15.9             | 302.0 ± 27.1              | 2.38 ± 0.15             |
| Medial femoral condyle            | 395.0 ± 25.8                | 41.6 ± 6.3*                     | 176.9 ± 19.8             | 306.5 ± 31.8              | 2.35 ± 0.19             |
|                                   | 411.1 ± 50.3                | 48.4 ± 2.5                      | 207.4 ± 15.3             | 285.2 ± 22.2              | 2.34 ± 0.09             |
|                                   | 377.1 ± 77.1                | 46.9 ± 3.4                      | 193.7 ± 9.5              | 283.6 ± 22.0              | 2.42 ± 0.07             |
| Lateral tibial plateau            | 297.1 ± 99.9*               | 37.4 ± 6.3**                    | 159.2 ± 10.7             | 310.7 ± 12.9              | 2.21 ± 0.05             |
|                                   | 395.0 ± 59.8                | 51.0 ± 3.2                      | 231.3 ± 16.5             | 296.6 ± 42.3              | 2.35 ± 0.16             |
|                                   | 356.3 ± 54.6                | 50.0 ± 3.2                      | 213.2 ± 16.5             | 294.0 ± 20.5              | 2.16 ± 0.09             |
| Medial tibial plateau             | 382.9 ± 65.0                | 41.2 ± 7.4**                    | 194.4 ± 8.4**            | 374.3 ± 90.5**            | 2.05 ± 0.30             |
|                                   | 403.4 ± 28.7                | 46.6 ± 3.8                      | 232.9 ± 22.3             | 378.2 ± 44.4**            | 2.01 ± 0.22             |
|                                   | 377.7 ± 45.3                | 52.0 ± 5.1                      | 231.8 ± 19.8             | 285.8 ± 40.0              | 2.25 ± 0.19             |
| Femoral groove                    | 320.6 ± 58.1**              | 28.3 ± 5.3**                    | 139.1 ± 12.8**           | 357.5 ± 72.6**            | 2.32 ± 0.11             |
|                                   | 399.6 ± 30.4*               | 41.4 ± 3.2                      | 178.9 ± 22.2             | 299.5 ± 31.4**            | 3.14 ± 0.21             |
|                                   | 346.1 ± 260                  | 39.3 ± 4.2                      | 148.4 ± 40.7             | 268.0 ± 93.0              | 2.03 ± 0.09             |
| Patella                           | 457.1 ± 568.0               | 61.9 ± 6.7                      | 230.7 ± 11.2             | 226.5 ± 37.2              | 2.65 ± 0.19             |
|                                   | 462.4 ± 54.5                | 65.9 ± 3.4                      | 248.9 ± 12.1             | 210.7 ± 22.6              | 2.66 ± 0.15             |
|                                   | 596.0 ± 45.7                | 62.7 ± 5.8                      | 235.8 ± 32.2             | 232.1 ± 35.6              |                       |
| Note: *Significant difference to the CNTRL group, *p < 0.05, **p < 0.01, ***p < 0.001; mean ± SD. #Significant difference between the ACLT and C-L group, #p < 0.05, ##p < 0.01, ###p < 0.001; mean ± SD. Abbreviations: ACLT, anterior cruciate ligament transection; C-L, contralateral; CNTRL, age-matched control.
compared to the C-L group (Table 3). The OARSI score was higher in the lateral ($p = 0.025$) and medial ($p = 0.042$) femoral condyle in the ACLT group compared to the C-L group. The OARSI score was also higher in the medial femoral condyle ($p = 0.006$) when compared to the CNTRL group 2 weeks after ACLT. As an indication of primary OA in the control group knees at the 2-week time point, we observed a higher OARSI score in the medial tibial plateau of the CNTRL group compared to the ACLT ($p = 0.001$) and C-L ($p < 0.001$) groups. The collagen orientation angle was slightly greater in the upper half of the lateral femoral condyle and the lateral tibial plateau, and slightly lower in the superficial medial femoral condyle of the 2-week ACLT group compared to the C-L and CNTRL groups (Figure 4). A smaller FCD content was observed in the superficial cartilage of the femoral condyles and medial tibial plateau (also see Figure S2), and in the upper half of the femoral groove and patella in the 2-week ACLT group compared to the FCD in the C-L group (Figure 5). In the 2-week ACLT group, the FCD content was smaller in the superficial cartilage of the lateral tibial plateau, medial femoral condyle, and femoral groove, and from the superficial to the middle layer of cartilage in the patella, as compared to those in the CNTRL group.

8 weeks after ACLT surgery, thicker articular cartilage was observed in the lateral tibial plateau in the ACLT group compared to the CNTRL group (50.48%, $p = 0.007$). The OARSI score was greater in the 8-week ACLT group of the lateral ($p = 0.004$) and medial ($p = 0.001$) femoral condyles, and lateral tibial plateau ($p = 0.029$) when compared to the C-L group, and to the CNTRL group ($p = 0.001$; $p < 0.001$; $p = 0.005$, respectively). When comparing the 8-week ACLT group to the C-L and CNTRL groups, there were minor changes in the collagen orientation angle in the articular cartilage of the medial femoral condyle, femoral groove and lateral tibial plateau, and changes in the superficial zone of the lateral femoral condyle (Figure 4). The FCD content was lower in the 8-week ACLT group than the C-L group in the superficial cartilage of the lateral femoral condyle, femoral groove and patella (also see Figure S2), and throughout the cartilage depth in the medial femoral condyle (Figure 5). When compared to the CNTRL group, the FCD content was lower in the 8-week ACLT group from the superficial up to the deep layer of cartilage in the medial femoral condyle, while the FCD content in the 8-week ACLT group of the lateral femoral condyle was smaller in the deep layer of cartilage. Furthermore, the FCD content in the C-L group was smaller than that in the CNTRL group in the superficial cartilage of the lateral femoral condyle and patella, as well as in the deep layer of cartilage in the lateral femoral condyle and medial tibial plateau.

4 | DISCUSSION

In this study, we examined concurrent changes in subchondral bone 3D structure and histology-based collagen organization and PG content of articular cartilage at different anatomical knee locations 2 and 8 weeks after ACLT surgery in a PTOA rabbit model. Our first hypothesis was confirmed since we observed degenerative changes...
in trabecular bone microstructure, cartilage composition, and histopathology as early as 2 weeks after the ACLT surgery, and these changes were tissue- and location-specific. During the study period, we observed novel dynamics of PTOA progression with bone degeneration becoming more severe from 2 to 8 weeks, while site-specific recovery of PG content was observed in the patella and femoral groove during the same observation period.

In our previous study the bone loss was observed in the medial femoral condyle of the ACLT group compared to the C-L group, without differences in the lateral femoral condyles 4 weeks after...

**FIGURE 4** The depth-wise collagen orientation angle in various knee locations 2 and 8 weeks after the surgery. Shaded color areas around the lines represent the confidence intervals (95% CI) and the straight dashed lines above the curves represent statistical difference ($p < 0.05$) between the groups. ACLT, anterior cruciate ligament transection (red curve); CI, confidence interval; C-L, contralateral (blue curve); CNTRL, age-matched control (black curve) [Color figure can be viewed at wileyonlinelibrary.com]

**FIGURE 5** The depth-wise optical density distributions in various knee locations 2 and 8 weeks after the surgery. Shaded color areas around the lines represent the confidence intervals (95% CI) and the straight dashed lines above the curves represent statistical difference ($p < 0.05$) between the groups. ACLT, anterior cruciate ligament transection (red curve); CI, confidence interval; C-L, contralateral (blue curve); CNTRL, age-matched control (black curve) [Color figure can be viewed at wileyonlinelibrary.com]
ACL surgery. In the current study bone loss occurred first in the lateral tibial plateau and femoral groove of the operated knees 2 weeks after the surgery, and later the bone loss spread to other anatomical locations. This demonstrates importance of studying multiple joint locations and time points, and indicates that the bone deterioration is progressive within 8 weeks after ACLT, supported by another study. In the evaluation of articular cartilage degeneration, increased OARSI scores were first observed in the femoral condyles, and later in the lateral tibial plateau. Thus, the response of the subchondral bone and articular cartilage to the ACLT appears to be tissue- and location-dependent. This supports our original hypothesis, and indicates that the most sensitive anatomical locations for structural changes in the subchondral bone during OA development are the lateral tibial plateau and femoral groove, while the cartilage in the femoral condyles exhibits the earliest changes. This is also supported by our earlier study on the biomechanical properties of cartilage and chondrocytes in post-ACLT rabbits, as well as the study on tibial plateaus in human OA.

The trabecular number was greater in the medial femoral condyle and femoral groove of operated compared to C-L knees 2 weeks after the ACLT, but not at the 8-week time point. Additionally, the subchondral plate was thinner in the lateral tibial plateau, femoral groove and patella of operated knees than the subchondral plate from the C-L knees 8 weeks after the surgery. In contrast, in rats subchondral plates were thinner and the trabecular number increased in the femoral condyles and medial tibial plateaus 4 and 10 weeks post-ACLT, without changes in trabecular thickness in the femoral condyles 4 weeks after the surgery and in the tibial plateaus both 4 and 10 weeks after the surgery. The different changes in subchondral plate and trabecular bone between rabbits and rats in PTOA indicate that these changes are probably species-dependent.

In this study, digital densitometry was used to measure the FCD, which reflects the PG content of articular cartilage. Our results provide novel insights on the dynamics of the PG content during OA progression. At the 2-week time-point, we observed a reduced PG content close to cartilage surface in both femoral condyles, especially when the ACLT group was compared to C-L group. There was even a greater loss of PG content in the same locations, especially in the medial compartment, at the 8-week time point, suggesting a progressive PG loss during the PTOA period. Furthermore, the PG content was clearly reduced in the top half of the femoral groove and patella 2 weeks after the surgery, whereas there was only some reduction of PG content in the superficial zone at these locations 8 weeks after the surgery. This result may indicate that there is a partial recovery of the PG content in articular cartilage. Additionally, a higher PG content was observed in the medial tibial plateau of C-L knees compared to controls 2 weeks after the surgery, which may indicate that modified loading could increase the PG synthesis in C-L knees. However, this effect was not observed at the 8-week time point.

In contrast to the PG content, only minor changes in collagen orientation were detected. It has been reported that collagen disorganization can occur in very early OA. Lack of changes in our collagen orientation may be attributed to methodology, as Mingalone et al. used Second Harmonic Generation imaging for collagen orientation assessment, and they did not quantify PG content as thoroughly as was done in this study. However, the results of our study suggest that PG loss occurs first, followed by changes in the collagen network, which is consistent with our previous work on articular cartilage, where the PG content decreased 3 days after a partial meniscectomy, without a corresponding alteration in collagen content and orientation.

The response of the subchondral plate thickness to the ACLT surgery was insensitivity, which could be attributed to contradictory changes in the thickness of subchondral bone plate and calcified cartilage. Calcified cartilage thickness has been found to increase during the initiation and development of OA in post-ACLT animal models and in our previous study. It is possible that the observed thickening of the calcified cartilage in the rabbit knees is accompanied with a thinning of the subchondral bone plate during the progression of OA, leading to unchanged subchondral plate thickness. Moreover, the asynchronous changes in calcified cartilage and subchondral bone during the progression of PTOA will also create challenges to the morphological analysis of the subchondral bone structure. To better assess the changes in calcified cartilage structure, more advanced imaging and image processing strategies are required to enable a µCT-based analysis of the calcified cartilage and the subchondral bone. These analyses may require the use of high-resolution µCT imaging, which will be one of our future goals.

This study has some limitations. The ACLT animal model we used in our study does not mimic perfectly human ACL injuries and subsequent tissue alterations, because clinically ACL rupture is often accompanied by bone bruises and meniscus tears, which might contribute to apoptosis, bone changes, and the progression of joint degeneration. The difference in tissue turnover and loading patterns between our animal model and humans produces challenges for the translation of our results to clinical applications. However, the fast tissue turnover in rabbits makes it easier to study OA within a short time period. The use of animal models also allows investigating OA in a controlled environment that is not possible with humans.

The resolution of the µCT imaging we used was moderate and was selected to allow comparisons to our previous studies. To detail further changes in the subchondral bone plate, a high-resolution µCT is needed to separate the subchondral bone plate porosity and calcified cartilage.

The OARSI score in the medial tibial plateau was greater in the CNTRL group compared to the C-L and ACLT groups at the 2-week time point. We can only speculate that this may be ascribed to the presence of spontaneous OA in the CNTRL group at the beginning of the experiments. We cannot exclude the possibility that spontaneous OA may have also occurred in the other groups. It has been reported that approximately 12.5% skeletally mature rabbits have spontaneous OA in hips and knees as early as 1 year of age.

The current study is limited to structural changes in the osseochondral unit at two time points after surgery. More time points are required to study time-dependent sequences of joint changes.
rupture. In our study, the lack of a sham control group makes it challenging to assess possible surgery-induced changes (pain, inflammatory response, and unloading) to the osteochondral unit. Thus, in future studies it could be relevant to add a sham-operation group.

5 | CONCLUSION

We conclude that our rabbit model of PTOA shows very early and progressive subchondral bone deterioration. PG loss is progressive in the femoral condyles, but in the medial tibial plateau, femoral groove and patella, there is a very early loss of PG followed by partly recovery. The tibial plateaus seem to be sensitive to changes in the bone morphology, while the femoral condyles are more sensitive to changes in cartilage composition and structure. These findings provide vital clues to the location-specific diagnosis and clinical study on human OA at the early stage.

ACKNOWLEDGMENTS

The authors would like to thank Andrew Sawatsky (from University of Calgary) for ACLT surgeries, sample collection and making time schedule, and Tarja Huhta (from University of Oulu) for the sample preparation. This project has received funding from the European Union’s Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 713645; the European Research Council (ERC) under the European Union’s Seventh Framework Programme (FP/2007-2013)/ERC Grant Agreement No 336267 and No 281180; Academy of Finland [grant numbers 286526, 303786, 324529]; Saastamoinen Foundation, Päivikki and Sakari Sohlberg Foundation; Finnish Cultural Foundation [grant number 00180796]; Finnish Cultural Foundation: North Savo Regional Fund [grant numbers 65171624, 00191044] and North Ostrobothnian Regional Fund [grant number 60172246]; The Canadian Institutes of Health Research [grant number FDN-143341], The Canada Research Chair Program [grant number 950-200955], and the Killam Foundation [grant number 10001203].

CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

AUTHOR CONTRIBUTIONS

This study was designed by Mikko A. J. Finnilä, Rami K. Korhonen, Simo J. Saarakkala and Walter Herzog. Mikko A. J. Finnilä, Lingwei Huang, Ilari Riihiöja, Simo Ojanen and Sanna Palosaari did data collection, processing and analysis. Statistical analysis: Mikko A. J. Finnilä, Lingwei Huang, Petri Tanska and Simo Ojanen. Manuscript drafting: Lingwei Huang. Manuscript editing and reviewing: all authors. Supervision: Mikko A. J. Finnilä, Petri Tanska, Heikki Kröger, Simo J. Saarakkala, Walter Herzog and Rami K. Korhonen. All the authors commented on the manuscript and accepted the final version of the manuscript before submission.

ORCID

Lingwei Huang https://orcid.org/0000-0002-0653-018X
Petri Tanska https://orcid.org/0000-0002-9684-6902
Simo Ojanen https://orcid.org/0000-0003-4386-0716
Simo J. Saarakkala https://orcid.org/0000-0003-2850-5484
Rami K. Korhonen https://orcid.org/0000-0002-3486-7855
Mikko A. J. Finnilä https://orcid.org/0000-0002-3348-5759

REFERENCES

1. Bijlsma JWJ, Berenbaum F, Lafeber FPJG. Osteoarthritis: an update with relevance for clinical practice. Lancet. 2011;377:2115-2126.
2. Anderson DD, Chubinskaya S, Guilak F, et al. Posttraumatic osteoarthritis: improved understanding and opportunities for early intervention. J orthopaed Res. 2011;29:802-809.
3. Tochigi Y, Vaseenon T, Heiner AD, et al. Instability dependency of osteoarthritis development in a rabbit model of graded anterior cruciate ligament transection. J Bone Joint Surg Am. 2011;93:640-647.
4. Batiste DL, Kirkley A, Laverty S, Thain LMF, Spouge AR, Holdsworth DW. Ex vivo characterization of articular cartilage and bone lesions in a rabbit ACL transection model of osteoarthritis using MRI and micro-CT. Osteoarthritis Cartilage. 2004;12:986-996.
5. Lotz M, Hashimoto S, Kuhn K. Mechanisms of chondrocyte apoptosis. Osteoarthritis Cartilage. 1999;7:389-391.
6. Yoshioka M, Coutts RD, Amiel D, Hacker SA. Characterization of a model of osteoarthritis in the rabbit knee. Osteoarthritis Cartilage. 1996;4:87-98.
7. Setton LA, Mow VC, Müller FJ, Pita JC, Howell DS. Mechanical properties of canine articular-cartilage are significantly altered following transection of the anterior cruciate ligament. J Orthopaed Res. 1994;12:451-463.
8. Saito M, Sasho T, Yamaguchi S, et al. Angiogenic activity of subchondral bone during the progression of osteoarthritis in a rabbit anterior cruciate ligament transection model. Osteoarthritis Cartilage. 2012;20:1574-1582.
9. Hashimoto S, Takahashi K, Amiel D, Coutts RD, Lotz M. Chondrocyte apoptosis and nitric oxide production during experimentally induced osteoarthritis. Arthritis Rheum. 1998;41:1266-1274.
10. Sah RL, Yang AS, Chen AC, et al. Physical properties of rabbit articular cartilage after transection of the anterior cruciate ligament. J Orthopaed Res. 1997;15:197-203.
11. Han SK, Ronkainen AP, Saarakkala S, et al. Alterations in structural macromolecules and chondrocyte deformations in lapine retro-patellar cartilage 9 weeks after anterior cruciate ligament transection. J Orthopaed Res. 2018;36:342-350.
12. Florea C, Malo MKH, Rautiainen J, et al. Alterations in subchondral bone plate, trabecular bone and articular cartilage properties of rabbit femoral condyles at 4 weeks after anterior cruciate ligament transection. Osteoarthritis Cartilage. 2015;23:414-422.
13. Ansari S, Khorsheid S, Karkhaneeh A. Engineering of gradient ossteochondral tissue: from nature to lab. Acta Biomater. 2019;87:41-54.
14. Radin EL, Paul IL, Tolkoff MJ. Subchondral bone changes in patients with early degenerative joint disease. Arthritis Rheum. 1970;13:400-405.
15. Zhen G, Wen C, Jia X, et al. Inhibition of TGF-beta signaling in mesenchymal stem cells of subchondral bone attenuates osteoarthritis. Nature Med. 2013;19:704-712.
16. Finnilä MAJ, Thevenot J, Aho OM, et al. Association between subchondral bone structure and osteoarthritis histopathological grade. J Orthopaed Res. 2017;35:785-792.

17. Chen Y, Hu Y, Yu YE, et al. Subchondral trabecular rod loss and plate thickening in the development of osteoarthritis. J Bone Mineral Res. 2018;33:316-327.

18. Klose-Jensen R, Hartlev LB, Boel LWT, et al. Subchondral bone turnover, but not bone volume, is increased in early stage osteoarthritic lesions in the human hip joint. Osteoarthritis Cartilage. 2015;23:2167-2173.

19. Karsdal MA, Leeming DJ, Dam EB, et al. Should subchondral bone turnover be targeted when treating osteoarthritis? Osteoarthritis Cartilage. 2008;16:638-646.

20. Bouchguya M, Alexander K, Norman Carmel E, et al. Use of routine clinical multimodality imaging in a rabbit model of osteoarthritis—part II: bone mineral density assessment. Osteoarthritis Cartilage. 2009;17:197-204.

21. Papaainnou N, Krallis N, Triantafillopoulos I, et al. Optimal timing of research after anterior cruciate ligament resection in rabbits. Contemp Top Lab Anim Sci. 2004;43:22-27.

22. Ojanen SP, Finnilä MAJ, Mäkelä JTA, et al. Anterior cruciate ligament rupture of the anterior cruciate ligament as models of post-traumatic osteoarthritis. Osteoarthritis Cartilage. 2018;70:281-287.

23. Mustonen AM, Käkelä R, Finnilä MAJ, et al. Anterior cruciate ligament transection alters the n-3/n-6 fatty acid balance in the lapine infrapatellar fat pad. Lipids Health Dis. 2019;18:67.

24. Hildebrand T, Ruegsegger P. A new method for the model-independent assessment of thickness in three-dimensional images. J Microsc. 1997;185:67-75.

25. Laverty S, Girard CA, Williams JM, Hunziker EB, Pritzker KPH. The OARSI histopathology initiative—recommendations for histological assessments of osteoarthritis in the rabbit. Osteoarthritis Cartilage. 2010;18(Suppl 3):S53-S65.

26. Király K, Lapveteläinen T, Arokoski J, et al. Application of selected cationic dyes for the semiquantitative estimation of glycosaminoglycans in histological sections of articular cartilage by microspectrophotometry. Histochem J. 1996;28:577-590.

27. Kiviranta I, Juvelin J, Säämänen AM, Helminen HJ. Microspectrophotometric quantitation of glycosaminoglycans in articular cartilage sections stained with Safranin O. Histochemistry. 1985;82:249-255.

28. Orozco GA, Tanska P, Florea C, Grodzinsky AJ, Korhonen RK. A novel mechanobiological model can predict how physiologically relevant dynamic loading causes proteoglycan loss in mechanically injured articular cartilage. Sci Rep. 2018;8:15599.

29. Spiesz EM, Thorpe CT, Thurner PJ, Screen HRC. Structure and collagen crimp patterns of functionally distinct equine tendons, revealed by quantitative polarised light microscopy (qPLM). Acta Biomater. 2018;70:281-292.

30. Rieppo J, Hallikainen J, Juvelin JS, Kiviranta I, Helminen HJ, Hyttinen MM. Practical considerations in the use of polarized light microscopy in the analysis of the collagen network in articular cartilage. Microsc Res Tech. 2008;71:279-287.

31. Ding M, Odgaard A, Hvid I, Hvid I. Changes in the three-dimensional microstructure of human tibial cancellous bone in early osteoarthritis. J Bone Joint Surg. 2003;85-B:906-912.

32. Maerz T, Kurdziel M, Newton MD, et al. Subchondral and epiphyseal bone remodeling following surgical transection and noninvasive rupture of the anterior cruciate ligament as models of post-traumatic osteoarthritis. Osteoarthritis Cartilage. 2016;24:698-708.

33. Hui Mingalone CK, Liu Z, Hollander JM, et al. Bioluminescence and second harmonic generation imaging reveal dynamic changes in the inflammatory and collagen landscape in early osteoarthritis. Lab Invest. 2018;98:656-669.

34. Ronkainen AP, Tanska P, Fick JM, Herzog W, Korhonen RK. Interrelationship of cartilage composition and chondrocyte mechanics after a partial meniscectomy in the rabbit knee joint—experimental and numerical analysis. J Biomech. 2019;83:65-75.

35. Hayami T, Pickarski M, Zuo Y, Wesolowski GA, Rodan GA, Duong LT. Characterization of articular cartilage and subchondral bone changes in the rat anterior cruciate ligament transection and meniscectomized models of osteoarthritis. Bone. 2006;38:234-243.

36. Aho OM, Finnilä M, Thevenot J, Saarakkala S, Lehenkari P. Subchondral bone histology and grading in osteoarthritis. PLOS One. 2017;12:e0173726.

37. Botter SM, van Osch GJVM, Waarsing JH, et al. Quantification of subchondral bone changes in a murine osteoarthritis model using micro-CT. Biomech. 2006;43:379-388.

38. Rytky SJO, Huang L, Tanska P, et al. Automated analysis of rabbit knee calcified cartilage morphology using micro-computed tomography and deep learning. Ann Biomed Eng. 2020.

39. Arzi B, Wilsner ER, Huey DJ, Kass PH, Hu J, Athanasiou KA. A proposed model of naturally occurring osteoarthritis in the domestic rabbit. Lab Anim. 2011;41:20-25.

40. Finnilä MA, Ojanen S, Saarakkala S, et al. Increased cartilage remodelling and impaired chondrocyte mechanotransduction in early post-traumatic osteoarthritis. Osteoarthritis Cartilage. 2017;25:S67-S68.

41. Elmosry S, Funakoshi T, Sasazawa F, Todoh M, Tadano S, Iwasaki N. Chondroprotective effects of high-molecular-weight cross-linked hyaluronic acid in a rabbit knee osteoarthritis model. Osteoarthritis Cartilage. 2014;22:121-127.

SUPPORTING INFORMATION
Additional Supporting Information may be found online in the supporting information tab for this article.

How to cite this article: Huang L, Rihioja I, Tanska P, et al. Early changes in osteochondral tissues in a rabbit model of post-traumatic osteoarthritis. J Orthop Res. 2021;1-12. https://doi.org/10.1002/jor.25009