Am J Transl Res 2023;15(2):847-857
www.ajtr.org /ISSN:1943-8141/AJTR0147265

Original Article

Microstructural changes of cartilage and subchondral bone in a guinea pig model of early- and middle-stage patellofemoral arthritis

Xuefeng Li[1*], Shihui Zhang[1*], Longlong Du[2], Fan Ping[3], Qimeng Gao[4], Yafei Liu[1]

[1]*Department of Orthopaedics, Honghui Hospital, Xi'an Jiaotong University, Xi'an 710054, Shaanxi, China;* [2]*Traditional Chinese Medicine, Honghui Hospital, Xi'an Jiaotong University, Xi'an 710054, Shaanxi, China;* [3]*Shaanxi University of Traditional Chinese Medicine School of Pharmacy, Xianyang 712046, Shaanxi, China;* [4]*The First Clinical Medical College of Shaanxi University of Traditional Chinese Medicine, Xianyang 712046, Shaanxi, China.* [*]*Equal contributors and co-first authors.*

Received October 26, 2022; Accepted November 27, 2022; Epub February 15, 2023; Published February 28, 2023

Abstract: Objective: Patellofemoral arthritis is a common type of knee osteoarthritis and a prime cause of anterior knee pain and disability. Most of the existing research on knee osteoarthritis focuses on tibial-femoral arthritis, while studies on patellofemoral arthritis are relatively rare. This study aims to observe changes in osteochondral and subchondral bone structure over time in the patella and femoral trochlea in an animal model of spontaneous patellofemoral arthritis. Methods: A total of 24 1-, 3- or 5-month-old healthy female Hartley guinea pigs were used for experiments. No intervention was applied, and the mechanical pain threshold was assessed prior to euthanasia. Bilateral knee joints were collected in the animals at the different ages, and the patellofemoral joints were taken to evaluate the bone microstructure of patellofemoral articular cartilage and subchondral bone by macroscopy, histopathology and micro-computed tomography (micro-CT). Results: There was a significant difference in the severity of femoral trochlea injury assessed by the Macro score between 5- and 1-month-old groups ($P<0.01$), as well as in patellar cartilage damage ($P<0.05$). The mechanical pain threshold of lower extremities in each group was statistically different between different age groups ($P<0.05$). The OARSI articular cartilage histopathological scores, including patella and femoral trochlea, were significantly different among 1-, 3- and 5-month-old groups. The 5-month-old group exhibited statistically lower values of bone volume/trabecular volume, trabecular number and trabecular thickness in the femoral subchondral bone and evidently higher structure model index than the 1-month-old group. Conclusions: This study demonstrated that 3- to 5-month-old female Hartley guinea pigs can develop early-to-mid-stage spontaneous patellofemoral arthritis that causes significant cartilage degeneration and loss of subchondral bone. In addition, the bone microarchitecture of the femur is more severely degraded.

Keywords: Patellofemoral arthritis, cartilage, subchondral bone, microstructure, guinea pig

Introduction

Knee osteoarthritis (KOA) [1], a high-incidence degenerative disorder featured by knee pain, and is associated with various risk factors such as age, sex, obesity and fatigue. The population aging and increased obesity rate have driven a rise in the prevalence of KOA, as well as the increasing burden of KOA on patients and society [2]. However, its pathogenic mechanism remains elusive, with relatively limited treatment options. Therefore, more research is needed to understand this disease.

The knee joint is a three-chambered structure comprising the patellofemoral joint and the medial and lateral tibiofemoral joints [3]. Traditional understanding of KOA focuses on tibiofemoral osteoarthritis (TFOA) [4]. However, patellofemoral osteoarthritis (PFOA) has attracted increasing clinical attention in recent years. Previous studies [5, 6] have shown that changes in PFOA, which accounts for about 65% of KOA patients, even precedes changes in TFOA in some cases. Symptomatic KOA is an important cause of knee pain [7], and its pain correlation is greater than TFOA. PFOA has a predilection for the middle-aged and elderly. The incidence of PFOA in Chinese people aged over 50 is increasing annually, with a 3-fold incidence in females than in males [8]. The first clinical sign of PFOA is anterior knee pain [9], which is aggra-

vated when the knee is bent during weight-bearing activities. Pathological changes include cartilage degeneration, subchondral bone remodeling and hyperosteogeny, etc. According to the 2016 patellofemoral pain consensus statement [10], PFOA may be a precursor of degenerative joint changes that eventuate in KOA. It is speculated that the main cause of PFOA is cartilage and subchondral bone-related lesions [11]. With the development of intelligent science and technology, the imaging research on KOA is becoming increasingly mature [12]. Micro-computed tomography (micro-CT), with the advantages of high-resolution images and two-dimensional (2D) and three-dimensional (3D) reconstruction of bone structure, has become the main means to detect the pathological morphology of bone tissue [13]. While micro-CT greatly assists in studying the underlying mechanism of KOA, most of the studies only demonstrated changes in bone micro-structure in TFOA but not the changes of cartilage and subchondral bone micro-structure in PFOA [14].

At present, the establishment of a KOA animal model has become relatively mature [15]. An animal model of PFOA is mainly constructed by inducing joint injury, but there are few spontaneous PFOA animal models that are characterized by slow disease progression, long study duration and variable outcomes [16-18]. Hartley guinea pigs, with pathological features similar to the degenerative changes of human osteoarthritis, are ideal animals to model the natural degeneration of human joints. The Osteoarthritis Research Society International (OARSI) has developed guidelines for histological examination of this species [18, 19]. Accordingly, this research aims to investigate the early and mid-term changes in patellofemoral cartilage and subchondral bone in female Hartley guinea pigs from different age groups. The novelty is to provide a basis for PFOA animal experiments and references for clinical treatment of PFOA by analyzing behavior changes, macromorphology and histopathology as well as micro-CT image of Hartley guinea pigs.

Material and methods

Experimental animals and groups

Twenty-four 1-, 3- or 5-month-old healthy female Hartley guinea pigs with a body weight of (350.0±8.3) g, (535.0±15.5) g, and (648.0±18.3) g, respectively, were used for experiments, with 8 pigs in each age group. All animals are provided by Xixian New area Jiadong New City Experimental Animal Farm (license number: SCXK (Shaanxi) 2018-001). The Experimental Animal Ethics Committee at Shaanxi University of Traditional Chinese Medicine ratified the experimental scheme. Animals were kept in a hygienic, well-ventilated environment and a 12-hour light/12-hour dark regime, with the temperature and relative humidity controlled at 22±2°C and 60%±20%, respectively, and were fed with special feed and vegetables. All animals were sacrificed within the corresponding months of age.

Experimental specimen collection and macroscopic observations

Animals were anesthetized and maintained with a mixture of 1.5 to 3% isoflurane and oxygen. The anesthetized animals were then immediately transferred to a carbon dioxide chamber for euthanasia. The knee joints of both hind limbs were stripped and washed with 0.9% sodium chloride solution (3B20070201, Qidu Pharmaceutical Industry), and the specimens were recorded by digital camera (Olympus, Japan). The patella and femoral trochlea were scored and summarized based on the Guingamp [20] naked eye lesion grading. The specific grades are as follows: 0: normal appearance; 1: yellowish discoloration of the cartilage surface; 2: erosion into the surface or the middle area; 3: downward erosion to the subchondral bone area; 4: large erosion, large area of subchondral bone exposure.

Mechanical pain threshold evaluation

The mechanical withdrawal threshold (MWT) of the guinea pigs was measured using a precision sensory evaluation instrument (VonFrey filament pain meter, Hong Kong Shengchang Co., Ltd.) [21]. To keep the environment quiet, the guinea pigs were placed in 30 cm × 20 cm × 25 cm glass compartments (with foot mats) to adapt to the environment for 30 min. According to the up & down method, VonFrey filaments (0.08, 0.2, 0.4, 0.6, 1.4, 2.0, 4.0, 6.0, 8.0, 15.0, 26.0 g, 60.0 g, 100.0, 180.0 g) were stimulated from 2.0 g (4.31 MN) to stimulate the center of both feet of guinea pigs vertically, and the fibers were bent to “C” or “S” for 4-10

seconds. Claw withdrawal or foot licking was considered a positive response. The mean MWT values were obtained after 3 repeated experiments at 0, 4, 8, 12, 16, 20 and 24 days, respectively.

Observation of cartilage pathological morphology

The left knee joint was fixed in 4% paraformaldehyde solution (AR1068, Wuhan Bosher Biological Technology Co., LTD., China) for about 48 h, followed by decalcification in 10% Ethylene Diamine Tetraacetic Acid (EDTA, XK-011-00008, Tianjin Hedong District Hongyan Reagent Factory, China) for about 6 weeks. The decalcified solution was replaced every other week when about half of the decalcification process was done. The joint was cut open with a sharp blade (guided by the trochlear groove), and then the two halves of the joint were put back into the decalcifying solution. After that, the tissue was taken out and rinsed properly with running water for 5 min, followed by three PBS rinses that lasted for 5 min each time. After fine cutting of the tissue around the knee joint, the knee joint was resected in the sagittal plane, dehydrated with gradient concentrations of ethanol, transparentized with xylene and embedded in paraffin. The specimens were cut into 3-μm slices, with at least 6 sections taken from each embedded wax block. After hematoxylin and eosin (H&E, Solabio biotechnology co., Ltd., China) staining, fuchsin fast green staining and toluidine blue staining, the cartilage structure was observed under 40-, 100- and 400-fold light microscopes. Capture 2.2.1 microscopic image analysis software was utilized to collect pictures. Meanwhile, OARSI articular cartilage histopathological scoring [22] (score range: 0-24) was used to evaluated the degree of cartilage lesions based on the three histologic staining approaches (H&E, fuchsin fast green and toluidine blue staining), with higher scores representing more serious damage.

Micro-CT scan imaging

The right knee joint was peeled and fixed with 4% paraformaldehyde solution for about 48 hours. After 30 min of rinsing with running water, the right knee joint was placed in 30% ethanol for 48 h, 50% ethanol for 24 h, and 70% ethanol for 2 hours, followed by immersion in 1% phosphotungstic acid solution for 1-3 days. Microtomography system (Skyscan1276, Bruker, Belgium) was used to image the patellofemoral joint. The parameters were 75 kV and 200 μA, and the resolution was 18 μm. The regions of interest (ROIs) of patellar cartilage and femoral trochlear cartilage were determined to be located in the medial center of the patella and the trochlear, with an area of a rectangle (1.2 × 2 mm), while the two ROIs of the subchondral bone were located in the cross section under the subchondral plate of the patella and the femur. After the scan, the parameters were analyzed and calculated with the matching software. In addition, cartilage analysis (bone volume, BV; bone surface, BS; trabecular thickness, Tb.Th) and subchondral bone analysis (bone volume/trabecular volume, BV/TV; trabecular number, Tb.N; trabecular thickness, Tb.Th; structure model index, SMI) were performed to study patellofemoral structure damage.

Statistical processing

The experimental results were statistically processed by GraphpadPrism 8.0 (GraphPad, Inc., San Diego, CA, USA). Data (denoted by $x \pm s$) were analyzed by one-way analysis of variance (ANOVA) plus Bonferroni post-hoc test and t-test to conduct multi-group and between-group comparisons, respectively. Data at different time points were compared by repeated measures ANOVA, followed by the Bonferroni post-hoc test. $P<0.05$ was the significance level.

Results

Macro score

In the 1-month-old group, the patellar surface and femoral trochlear surface cartilage were smooth, translucent, regular and non-eroding. The patellar surface in the 3- and 5-month-old groups became smooth and yellow, with no crack. The condition in the 5-month-old group became worse, with smooth patellar surface cartilage, flat edge, and obviously thin and rough femoral trochlear surface, while there was partial erosion and no gloss (**Figure 1**). The macroscopic score of patellar facial cartilage was similar in the 3- and 5-month-old groups ($P>0.05$), which was significantly increased when compared with the 1-month-old group

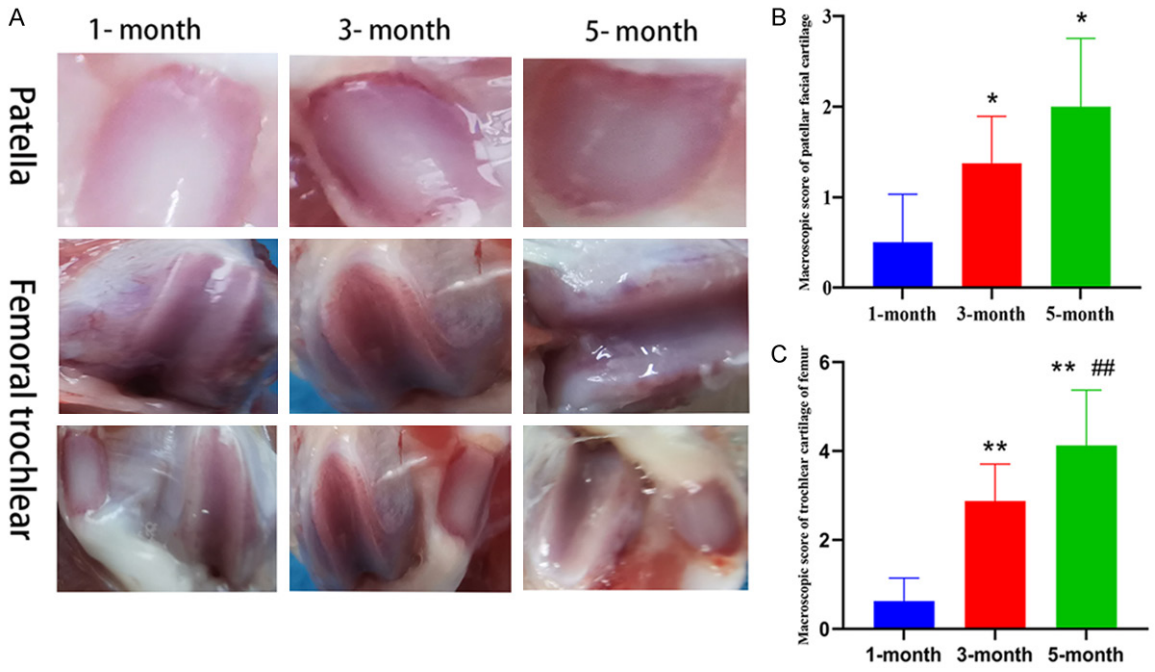

Figure 1. Macroscopic observation and macroscopic scores of the patellofemoral joint of guinea pigs in each group. A: Macroscopic observation; B: Macroscopic score of patellar facial cartilage; C: Macroscopic score of trochlear cartilage of the femur. Compared to the 1-month-old group, *P<0.05, **P<0.01; Compared to the 3-month-old group, ##P<0.01.

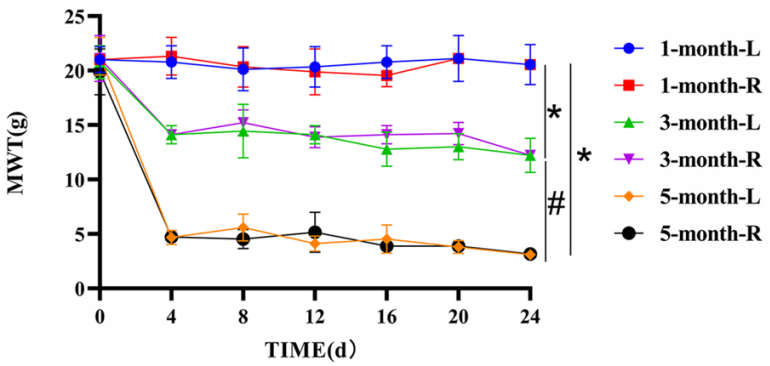

Figure 2. MWT of the hind limbs of guinea pigs in each group (*P<0.05; #P<0.05). MWT: Mechanical Withdrawal Threshold.

(P<0.05). The 5-month-old group showed higher macroscopic scores of the femoral trochlear cartilage than the 1- and 3-month-old groups (P<0.05).

Evaluation of mechanical pain threshold

On the first day, there was no significant difference in the MWT of guinea pigs among groups (P>0.05), but there was significant difference in MWT of lower extremities in 5-, 3- and 1-month-old groups from the fourth day (P<0.05). From the fourth day, MWT at each time point in the 3- and 5-month-old groups was significantly lower than that in the 1-month-old group, respectively (P<0.05). Meanwhile, no significant difference was observed in MWT between left and right lower extremities among groups (P>0.05) (**Figure 2**).

Observation of pathological morphology of cartilage tissue

The paraffin sections of the sagittal section of the knee joint of guinea pigs in each group were subjected to H&E, fast green and toluidine blue staining microscopically. In the 1-month-old group, the cartilage on the patellar and the trochlear surfaces of the femur was intact, the morphology of chondrocytes was normal, the arrangement was uniform and regular, and the surface staining of cartilage was uniform, with no crack and visible tide line (**Figures 3A**, **4A**, **5A**). In the 3-month-old group, there were enlarged chondrocytes and clustered chondro-

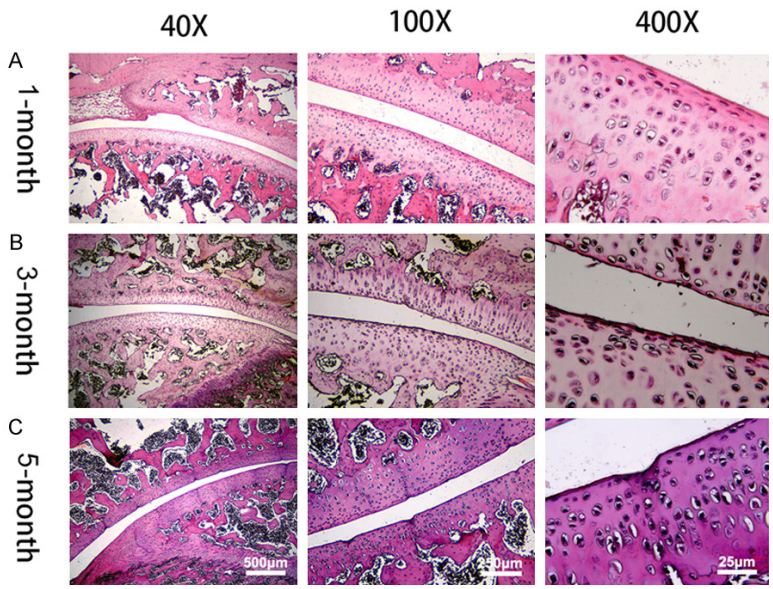

Figure 3. Hematoxylin-eosin staining of patellofemoral cartilage tissue from Hartley guinea pigs. A: 1-month-old; B: 3-month-old; C: 5-month-old.

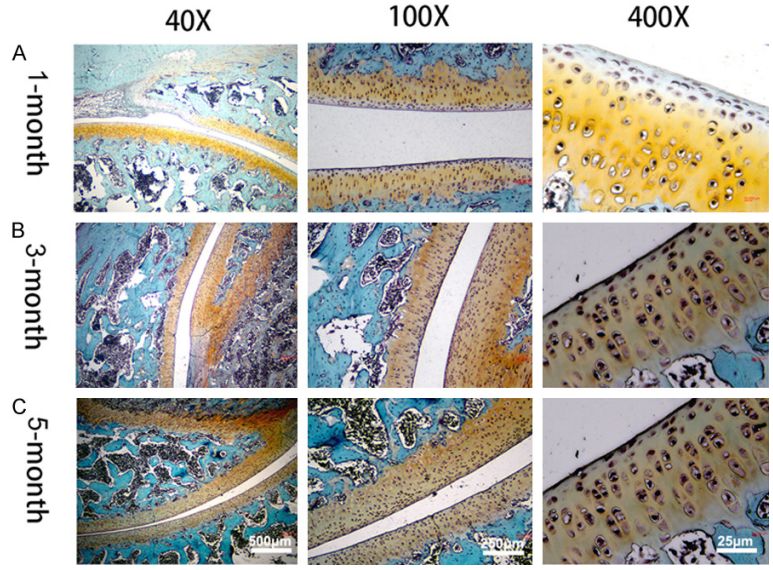

Figure 4. Safranin-fast green staining of patellofemoral cartilage tissue from Hartley guinea pigs. A: 1-month-old; B: 3-month-old; C: 5-month-old.

cytes on the surface of patellar cartilage, uneven surface of cartilage on the trochlear surface of femur, uneven staining or absence of cartilage on the surface of cartilage and uneven density of cells (**Figures 3B**, **4B**, **5B**). In the 5-month-old group, the surface of patellar cartilage was damaged, the surface was rough, the shape of chondrocytes was irregular, and the surface of cartilage on the trochlear surface of femur was punctate or sunken, with partial missing of the matrix staining, partial interruption of the subchondral bone trabeculae, and vertical cracks extending to the middle layer (**Figures 3C**, **4C**, **5C**). The OARSI scores with toluidine blue staining are shown in **Figure 6**. It can be found that the OARSI articular cartilage histopathological score of patellar cartilage and trochlear cartilage of the femur increased gradually over time (**Figure 6**). The OARSI of trochlear cartilage of femur was significantly increased in the 5-month-old group as compared with that in the 1- and 3-month-old groups ($P<0.01$) (**Figure 6**).

Micro-CT measurements of cartilage and subchondral bone

Figure 7 presents the cartilage (**Figure 7A** and **7B**), subchondral bone detection selection areas (**Figure 7C** and **7D**) and overall image of the ROIs (**Figure 7E**). Three-dimensional reconstruction and sectional maps of subchondral bone in different age groups are shown in **Figure 8**.

Micro-CT measurement results of cartilage and subchondral bone in different age groups: BV and Tb.Th of patellar cartilage were not statistically different among the three age groups ($P>0.05$), but there were significant differences in trochlear cartilage of femur BV and Tb. ($P<0.05$, **Figure 9A-D**).

BV/TV, Tb.N and Tb.Th of subchondral bone of the patella and femur in the 5-month-old group were decreased, while SMI was statistically elevated, but there was no significant difference in the degree of decline in BV/TV and

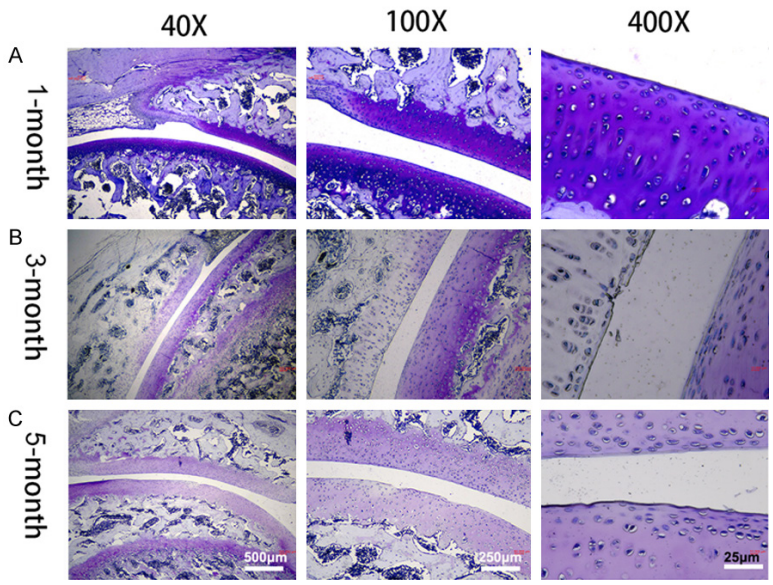

Figure 5. Toluidine blue staining of patellofemoral cartilage tissue from Hartley guinea pig. A: 1-month-old; B: 3-month-old; C: 5-month-old.

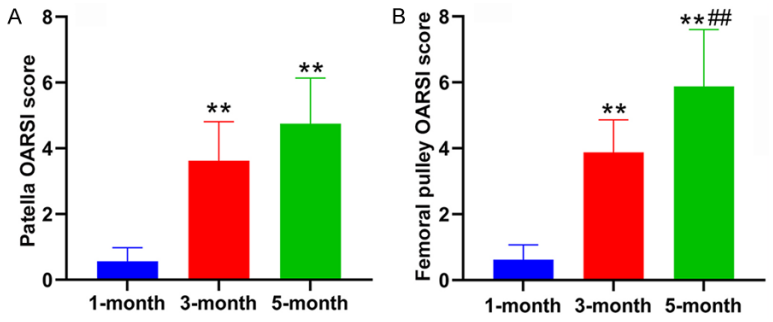

Figure 6. OARSI articular cartilage histopathological scores of guinea pig patella and femoral trochlea in each group. A: Patella OARSI score; B: Femoral trochlea OARSI score. Compared with the 1-month-old group, $^{**}P<0.01$; Compared with the 3-month-old group, $^{\#\#}P<0.01$. OARSI: Osteoarthritis Research Society International.

TB.N of the subchondral bone of patella. On the contrary, BV/TV, Tb.Th of the subchondral bone of femur was significantly decreased while SMI increased in 5-month-old group ($P<0.05$, **Figure 9E-L**).

Discussion

Osteoarthritis (OA), a common degenerative musculoskeletal disorder, is one of the main factors affecting the activity ability of the elderly [1]. The incidence of primary PFOA of 5.8/100,000 is an ever-higher incidence among young individuals [23]. Cartilage injury and subchondral osteosclerosis are currently the main research directions of pathological changes in PFOA [12]. In this study, we successfully developed an experimental model of early- and mid-stage PFOA that is characterized by cartilage injury deterioration and subchondral bone loss with age. There are a variety of methods to evaluate the success of PFOA experimental models: first, the statistical results of Guingamp macroscopic score and mechanical pain threshold support the successful establishment of the model; second, histology shows rough and damaged surfaces of the patella and femoral trochlear cartilage, obviously uneven staining associated with hypertrophy and disordered chondrocytes, and increased pathological scores of OARSI cartilage with age, consistent with the pathological manifestations of early- and middle-stage OA; third, micro-CT confirms changes in cartilage thickness and volume and deterioration of subchondral trabecular microstructure. In addition, this is the first study to investigate the relationship between spontaneous PFOA patellar and femoral trochlear cartilage and subchondral bone lesions.

Animal models involving PFOA are mainly induced by surgery. TAKAHASHI et al. [24] established an OA model through injection of different doses of MIA into the knee joint cavity of guinea pigs. It was found that with similar histopathological changes, the OA progression of the patellofemoral joint was slower than that of the tibiofemoral joints. Clark et al. [25] observed the histopathological changes of patellofemoral articular cartilage in cats after anterior cruciate ligament transection (ACL-T), and found mild OA changes at 4 months. In the study of Chang et al. [26], erosion of the surface of patellofemoral articular cartilage was detected 4 weeks after ACLT in rabbits. In addi-

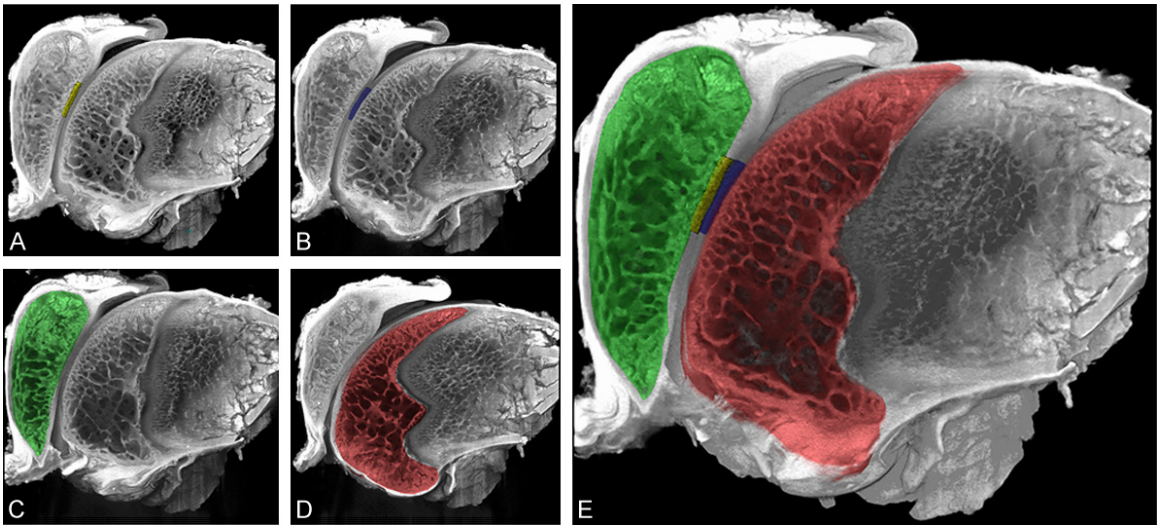

Figure 7. Micro-CT measurements of the region of interest. A: Patellar cartilage; B: Femoral cartilage (the yellow circled area is the patellar cartilage, and the blue circled area is the trochlear cartilage of the femur); C: Patellar subchondral bone; D: Femoral subchondral bone (the green delineated area is the subchondral bone of the patella, and the red delineated area is the subchondral bone of the femur); E: Overall image of the region of interest.

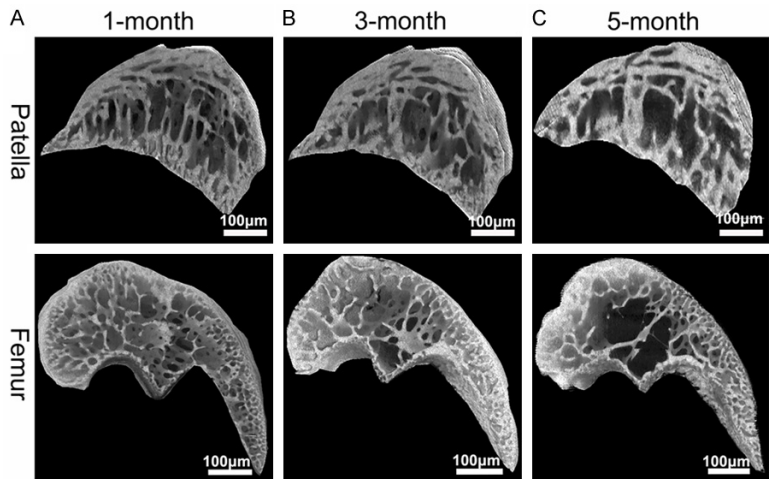

Figure 8. Micro-CT 3D reconstruction image. A: 1-month-old patellar subchondral bone and femoral subchondral bone; B: 3-month-old femoral subchondral bone and femoral subchondral bone; C: 5-month-old femoral subchondral bone and femoral subchondral bone.

tion, Bei et al. [27] used patellar ligament shortening to establish a PFOA model, and found that patellar cartilage and femoral trochlear load-bearing area cartilage were significantly damaged in the model group. In this study, female Hartley guinea pigs with primary OA were used as animal models, without related intervention. Most importantly, this model will not cause damage to the articular cavity and its surrounding tissues, which is better than previous PFOA models and can approximately reflect the natural development of human PFOA.

The degeneration of articular cartilage is one of the main characteristics of PFOA affecting joints [28]. At present, cartilage degeneration in OA animal models is mainly based on macroscopic evaluation of bone tissue and histopathological sections, which has some limitations. Combined with micro-CT joint 3D reconstruction, specimens can be evaluated longitudinally, subtle bone structural lesions can be easily found in the early stage, and OA disease progression can be detected more sensitively [29]. In this study, the ROI cartilage bone volume and bone thickness were measured by micro-CT. It was found that the index value of the 5-month-old group was markedly lower compared with the 1-month-old group, and the trochlear thickness of the femur decreased significantly with age, but there was no difference in the bone area in the three-month-old group compared with the 1-month-old group, which may be affected by multiple separate cross-sectional sets of 2D analysis. The histopathological scores of OARSI

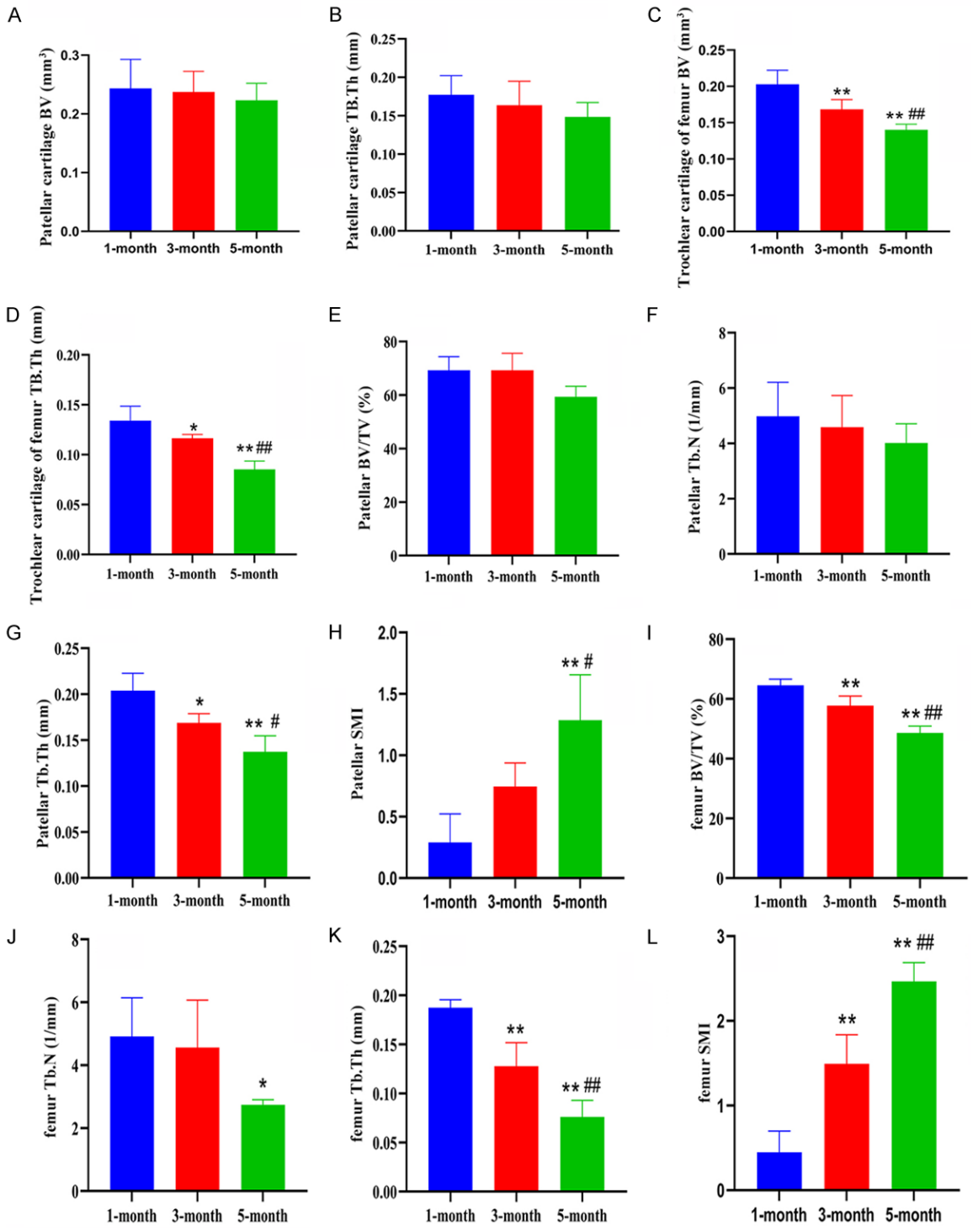

Figure 9. Micro-CT subchondral bone analysis. A: Patellar cartilage bone volume (BV); B: Patellar trochlear trabecular thickness (Tb.Th); C: Femoral trochlear cartilage BV; D: Femoral cartilage Tb.Th; E: Patellar bone volume/trabecular volume (BV/TV); F: Patellar trabecular number (Tb.N); G: Patellar Tb.Th; H: Patellar structure model index (SMI); I: Femoral BV/TV; J: Femoral Tb.N; K: Femoral Tb.Th; L: Femoral SMI. Compared with the 1-month-old-group, $^{*}P<0.05$, $^{**}P<0.01$; Compared with the 3-month-old group, $^{\#}P<0.05$, $^{\#\#}P<0.01$.

cartilage of patellar cartilage and femoral cartilage in the 1-month-old group were less than 1 (normal cartilage), while those in 3- and 5-month-old groups were significantly higher,

which was similar to that in guinea pigs with spontaneous OA in different age groups [30]. Among them, there was a large area of cartilage erosion and cell loss in the femoral trochlear weight-bearing area in the 5-month-old guinea pigs, but no significant destruction was found in patellar cartilage, which is supported by the clinical study on PFOA [31]. Some scholars have reported more serious cartilage destruction in 7-month-old guinea pigs than in 3-month-old guinea pigs [32]. However, the patellofemoral articular cartilage was only studied in the sagittal plane, and a comprehensive histopathological analysis of articular cartilage on other horizontal planes is needed in future studies.

In addition to cartilage degeneration, PFOA is characterized by abnormal bone remodeling of the subchondral bone that is featured by a high bone turnover rate and bone mass loss; high bone turnover will damage the microstructure of subchondral bone, which plays a vital part in OA progression [33]. The changes of BV/TV of subchondral bone in 5-month-old guinea pigs from another study [34] is consistent with the results of this study, and the change difference may be due to the different anatomic location of ROIs. Some scholars have detected the OA of female Hartley guinea pigs at the age of 1, 3, 6 and 9 months, and found that the BV/TV of the tibia decreased gradually over time, which supports our findings [35]. This indicates that the patellar articular subchondral bone has abnormal bone turnover and that the microstructure of subchondral bone has been destroyed in the early and middle stages of PFOA, which further confirms the important role of the subchondral bone in the onset of PFOA.

However, this study still has room for improvement. First, the establishment of a guinea pig model is not easily comparable to PFOA in humans, warranting clinical exploration. Second, this experiment only studied alterations in cartilage and subchondral bone in early- and mid-stage PFOA, while spontaneous OA is a relatively long-term process. In the future, a complete PFOA model should be constructed to expand the age groups of the experimental animals. Third, abnormal joint load will also cause changes in OA, so we should further compare the pathological alterations in patellar articular cartilage and subchondral bone, and explore the influencing mechanism of cartilage and subchondral bone on PFOA.

Conclusion

In conclusion, this study confirmed that 3-5-month-old female Hartley guinea pigs could be used for an animal model of early- and mid-stage spontaneous PFOA, and that both their cartilage and subchondral bone deteriorated gradually with the passage of time, especially the femur.

Disclosure of conflict of interest

None.

Address correspondence to: Yafei Liu, Department of Orthopaedics, Honghui Hospital, Xi'an Jiaotong University, Xi'an 710054, Shaanxi, China. Tel: +86-029-33341362; E-mail: Liuyafei9521@126.com

References

[1] Bijlsma JW, Berenbaum F and Lafeber FP. Osteoarthritis: an update with relevance for clinical practice. Lancet 2011; 377: 2115-2126.

[2] Zhang Z, Huang C, Jiang Q, Zheng Y, Liu Y, Liu S, Chen Y, Mei Y, Ding C and Chen M. Guidelines for the diagnosis and treatment of osteoarthritis in China (2019 edition). Ann Transl Med 2020; 8: 1213.

[3] Lin W, Kang H, Dai Y, Niu Y, Yang G, Niu J, Li M and Wang F. Early patellofemoral articular cartilage degeneration in a rat model of patellar instability is associated with activation of the NF-κB signaling pathway. BMC Musculoskelet Disord 2021; 22: 90.

[4] Zeng C, Wang H, Wu Z, Wang Y, Hu Y and Lei G. Interpretation of Chinese clinical practice guideline for patellofemoral osteoarthritis (2020 edition). Chin J Orthop 2021; 129-132.

[5] Xu J, Zhou W and Luo X. Visual analysis of patellofemoral pain syndrome research hotspots and content. Chin J Tissue Eng 2022; 26: 1877.

[6] van Middelkoop M, Bennell KL, Callaghan MJ, Collins NJ, Conaghan PG, Crossley KM, Eijkenboom JJFA, van der Heijden RA, Hinman RS, Hunter DJ, Meuffels DE, Mills K, Oei EHG, Runhaar J, Schiphof D, Stefanik JJ and Bierma-Zeinstra SMA. International patellofemoral osteoarthritis consortium: consensus statement on the diagnosis, burden, outcome measures, prognosis, risk factors and treatment. Semin Arthritis Rheum 2018; 47: 666-675.

[7] Crossley K and Hinman R. The patellofemoral joint: the forgotten joint in knee osteoarthritis. Osteoarthritis Cartilage 2011; 19: 765-767.

[8] Li Z, Liu Q, Zhao C, Gao X, Han W, Stefanik JJ, Jin Q, Lin J and Zhang Y. High prevalence of patellofemoral osteoarthritis in China: a multi-

center population-based osteoarthritis study. Clin Rheumatol 2020; 39: 3615-3623.
[9] Gaitonde DY, Ericksen A and Robbins RC. Patellofemoral pain syndrome. Am Fam Physician 2019; 99: 88-94.
[10] Schiphof D, van Middelkoop M, de Klerk BM, Oei E, Hofman A, Koes BW, Weinans H and Bierma-Zeinstra SM. Crepitus is a first indication of patellofemoral osteoarthritis (and not of tibiofemoral osteoarthritis). Osteoarthritis Cartilage 2014; 22: 631-638.
[11] Eijkenboom J, Waarsing J, Oei E, Bierma-Zeinstra S and van Middelkoop M. Is patellofemoral pain a precursor to osteoarthritis? Patellofemoral osteoarthritis and patellofemoral pain patients share aberrant patellar shape compared with healthy controls. Bone Joint Res 2018; 7: 541-547.
[12] Bayramoglu N, Nieminen MT and Saarakkala S. Machine learning based texture analysis of patella from X-rays for detecting patellofemoral osteoarthritis. Int J Med Inform 2022; 157: 104627.
[13] Du Longlong YP, Yang W, Li X and Gao Q. Advantages of micro CT in three-dimensional reconstruction of specimens and its application in animal models of osteoarthritis. Chin J Tissue Eng Res 2022; 26: 1931.
[14] Li J, Su Y and Bai D. Morphological characteristics of subchondral bone in a mouse model of early osteoarthritis. Chin J Tissue Eng Res 2022; 26: 1692.
[15] Kim JE, Song DH, Kim SH, Jung Y and Kim SJ. Development and characterization of various osteoarthritis models for tissue engineering. PLoS One 2018; 13: e0194288.
[16] Naruse K, Urabe K, Jiang SX, Uchida K, Kozai Y, Minehara H, Mikuni-Takagaki Y, Kashima I and Itoman M. Osteoarthritic changes of the patellofemoral joint in STR/OrtCrlj mice are the earliest detectable changes and may be caused by internal tibial torsion. Connect Tissue Res 2009; 50: 243-255.
[17] Salo PT, Seeratten RA, Erwin WM and Bray RC. Evidence for a neuropathic contribution to the development of spontaneous knee osteoarthrosis in a mouse model. Acta Orthop Scand 2002; 73: 77-84.
[18] McCoy A. Animal models of osteoarthritis: comparisons and key considerations. Vet Pathol 2015; 52: 803-818.
[19] Cook JL, Kuroki K, Visco D, Pelletier JP, Schulz L and Lafeber FP. The OARSI histopathology initiative-recommendations for histological assessments of osteoarthritis in the dog. Osteoarthritis Cartilage 2010; 18 Suppl 3: S66-S79.
[20] Guingamp C, Gegout-Pottie P, Philippe L, Terlain B, Netter P and Gillet P. Mono-iodoacetate-induced experimental osteoarthritis. A dose-response study of loss of mobility, morphology, and biochemistry. Arthritis Rheum 1997; 40: 1670-1679.
[21] Chaplan SR, Bach FW, Pogrel J, Chung J and Yaksh T. Quantitative assessment of tactile allodynia in the rat paw. J Neurosci Methods 1994; 53: 55-63.
[22] Pritzker KP, Gay S, Jimenez SA, Ostergaard K, Pelletier JP, Revell PA, Salter D and Van den Berg WB. Osteoarthritis cartilage histopathology: grading and staging. Osteoarthritis Cartilage 2006; 14: 13-29.
[23] Kaymaz B, Atay OA, Ergen FB, Mermerkaya MU, Olgun ZD, Atesok K and Doral MN. Development of the femoral trochlear groove in rabbits with patellar malposition. Knee Surg Sports Traumatol Arthrosc 2013; 21: 1841-1848.
[24] Takahashi I, Matsuzaki T, Kuroki H and Hoso M. Induction of osteoarthritis by injecting monosodium iodoacetate into the patellofemoral joint of an experimental rat model. PLoS One 2018; 13: e0196625.
[25] Clark A, Leonard T, Barclay L, Matyas J and Herzog W. Opposing cartilages in the patellofemoral joint adapt differently to long-term cruciate deficiency: chondrocyte deformation and reorientation with compression. Osteoarthritis Cartilage 2005; 13: 1100-1114.
[26] Chang NJ, Shie MY, Lee KW, Chou PH, Lin CC and Chu CJ. Can early rehabilitation prevent posttraumatic osteoarthritis in the patellofemoral joint after anterior cruciate ligament rupture? Understanding the pathological features. Int J Mol Sci 2017; 18: 829.
[27] Bei MJ, Tian FM, Xiao YP, Cao XH, Liu N, Zheng ZY, Dai MW, Wang WY, Song HP and Zhang L. Raloxifene retards cartilage degradation and improves subchondral bone micro-architecture in ovariectomized guinea pigs with patella baja-induced-patellofemoral joint osteoarthritis. Osteoarthritis Cartilage 2020; 28: 344-355.
[28] Bei M, Tian F, Liu N, Zheng Z, Cao X, Zhang H, Wang Y, Xiao Y, Dai M and Zhang L. A novel rat model of patellofemoral osteoarthritis due to patella baja, or low-lying patella. Med Sci Monit 2019; 25: 2702.
[29] Zamli Z, Robson Brown K, Tarlton JF, Adams MA, Torlot GE, Cartwright C, Cook WA, Vassilevskaja K and Sharif M. Subchondral bone plate thickening precedes chondrocyte apoptosis and cartilage degradation in spontaneous animal models of osteoarthritis. Biomed Res Int 2014; 2014: 606870.
[30] Wang T, Wen CY, Yan CH, Lu WW and Chiu KY. Spatial and temporal changes of subchondral bone proceed to microscopic articular cartilage degeneration in guinea pigs with spontaneous osteoarthritis. Osteoarthritis Cartilage 2013; 21: 574-581.

[31] Ryu J, Saito S and Yamamoto K. Changes in articular cartilage in experimentally induced patellar subluxation. Ann Rheum Dis 1997; 56: 677-681.
[32] Yan JY, Tian FM, Wang WY, Cheng Y, Xu HF, Song HP, Zhang YZ and Zhang L. Age dependent changes in cartilage matrix, subchondral bone mass, and estradiol levels in blood serum, in naturally occurring osteoarthritis in guinea pigs. Int J Mol Sci 2014; 15: 13578-13595.
[33] Goldring MB and Goldring SR. Articular cartilage and subchondral bone in the pathogenesis of osteoarthritis. Ann N Y Acad Sci 2010; 1192: 230-237.
[34] Radakovich LB, Marolf AJ, Shannon JP, Pannone SC, Sherk VD and Santangelo KS. Development of a microcomputed tomography scoring system to characterize disease progression in the Hartley guinea pig model of spontaneous osteoarthritis. Connect Tissue Res 2018; 59: 523-533.
[35] Yan JY, Tian FM, Wang WY, Cheng Y, Song HP, Zhang YZ and Zhang L. Parathyroid hormone (1-34) prevents cartilage degradation and preserves subchondral bone micro-architecture in guinea pigs with spontaneous osteoarthritis. Osteoarthritis Cartilage 2014; 22: 1869-1877.