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Effect of Posterior Cruciate Ligament Rupture on Biomechanical and Histological Features of Lateral Femoral Condyle

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

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Background: The aim of this study was to investigate bone mineral density (BMD) and the biomechanical and histological effects of posterior cruciate ligament (PCL) rupture on the lateral femoral condyle.

Material/Methods: Strain on different parts of the lateral femoral condyle from specimens of normal adult knee joints, including 12 intact PCLs, 6 ruptures of the anterolateral bundle, 6 ruptures of the postmedial bundle, and 12 complete ruptures, was tested when loaded with different loads on the knee at various flexion angles. Lateral femoral condyles were also collected randomly from both the experimental side in which the PCLs were transected and the control side from 4 sets of 12 matched-mode pairs of rabbits at 4, 8, 16, and 24 weeks after surgery, and their BMD and morphological and histological changes were observed.

Results: Partial and complete rupture of the PCL may cause an abnormal load on all parts of the lateral femoral condyle with any axial loading at all positions. Noticeable time-dependent degenerative histological changes of the lateral femoral condyle were observed in the rabbit model of PCL rupture. All of the PCL rupture groups had a higher expression of matrix metalloproteinase-7 (MMP-7) and collagen type II than the control group at all time points ($P<0.05$), but no significant difference in BMD ($P>0.05$).

Conclusions: Rupture of the PCL may trigger a coordinated response of lateral femoral condyle degeneration in a time-dependent manner, to which the high level of expression of MMP-7 and collagen type II could contribute.

MeSH Keywords: Bone Density • Collagen Type II • Matrix Metalloproteinase 7 • Posterior Cruciate Ligament

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Background

The posterior cruciate ligament (PCL) is the primary restraint to tibial posterior draw, contributing approximately 90% of the resistance across most of the arc of knee flexion [1], and is widely accepted as being classified into 2 components: the anterolateral bundle (ALB) and the posteromedial bundle (PMB) [2]. The incidence of PCL damage reported by epidemiologic studies ranges from 3% to 44% in acute knee injuries [3–5], among which an isolated PCL injury represents almost 17% [6]. The lateral femoral condyle is one of the cruciate components of the knee, the main movement of which is the extension and flexion of the femur in the sagittal plane. The patellofemoral joint comprises the lateral femoral condyle and the patella, and the strain on the lateral femoral condyle increases during knee movement [7]. Articular cartilage is prone to injury when the PCL is ruptured, and that in the femoral condyle suffers the most serious damage. Previous evidence supports that the PCL injury may result in degenerative changes of the lateral femoral condyle [8,9]. Specifically, after the injury, the knee joint is unstable and the normal movement of the knee joint is further affected. In addition, secondary injury of the joint cartilage may occur under the stimulation of adverse stress, and thus lead to the induction of osteoarthritis (OA) in the late stage. Multiple previous studies have focused on exploring the association of anterior cruciate ligament or PCL injury with the incidence of OA. For example, Sheldbourne et al. found in their research that there might be an occurrence of medial OA in PCL injury patients in long-term follow-up. Furthermore, reconstruction of the cruciate ligament helps restore anatomical and biomechanical properties of the cruciate ligament, necessary to maintain anteroposterior and anteromedial knee joint stability [10,11]. Due to the high occurrence rate of knee injuries observed nowadays, factors such as cruciate ligament elasticity, ligament acapsular structure stability, knee joint range of movement, and stress level of knee joint muscles are critical for maintaining normal movement abilities [11,12]. The loading and stress borne by the lateral femoral condyle may be redistributed after the injury, resulting in chronic injury to the lateral femoral condyle. For example, PCL rupture has been shown to lead to characteristic furrow-shaped cartilage damage in the lateral femoral condyle [9]. Importantly, furrow-shaped damage is the characteristic feature of cartilage damage in the femoral condyle induced by the unstable knee [1]. PCL rupture may initiate compensatory mechanisms by other adjacent articular structures to maintain normal knee function, which result in their degradation and, finally, OA of the knee [13,14]. We believe that the influence and mechanism of a partial rupture of the PCL on the lateral femoral condyle has not been sufficiently elaborated to date.

The lateral femoral condyle consists of subchondral bone and a superficial covering of cartilage. A balance between bone formation and the resorption of subchondral bone is maintained under optimum strain. Long-term overload may cause obstruction of the cartilage matrix synthesis and result in degenerative diseases [15]. The acceleration of joint degeneration continues in a vicious circle. Investigation of the biomechanical and histological changes of PCL rupture on the lateral femoral condyle is therefore critical to reveal the mechanism of secondary injury to articular cartilage after PCL rupture and to guide the clinical treatment of PCL injury. Bone mineral density (BMD) is a vital indicator of bone quality and can be used to evaluate biomechanical changes in subchondral bone [16]. In this regard, the measurement of BMD of the lateral femoral condyle can indirectly reflect the progression of OA due to biomechanical changes of the knee following fracture. In addition, articular cartilage is composed of many extracellular matrix and cartilage cells. The predominant effect of the extracellular matrix is to maintain the stability of the surrounding environment and to maintain the physiological function and biomechanical properties of articular cartilage. The existence of cartilage cells make articular cartilage become an occlusor possessing strength, hardness, toughness, and flexibility. Matrix metalloproteinase-7 (MMP-7) and collagen type II are widely accepted biomarkers of cartilage damage and degradation [17,18], and a study of their expression levels in a model of the lateral femoral condyle with a PCL rupture may increase our understanding of lateral femoral condyle degradation induced by PCL injury and the pathogenesis of OA [19].

In our research, specially designed strain gauges were placed on the anterior, middle, and posterior parts of the lateral femoral condyle in intact PCLs, PCLs with a partial rupture, and cadaveric knees with complete ruptures. The strain on these parts was then recorded under different conditions of knee flexion angles and axial loads. At the same time, an established rabbit model of a PCL rupture was used to observe the gross and BMD changes of the lateral femoral condyle cartilage. Expression of MMP-7 and collagen type II in the lateral femoral condyle cartilage were further studied by hematoxylin and eosin (HE) staining and immunohistochemical methods. The purpose of this study was to determine whether a PCL rupture could cause cartilage degradation in the lateral femoral condyle and to explore its biological mechanisms.

Material and Methods

Subjects

The study was approved by the Ethics Committee at Xiangya Hospital, Central South University (Grant number: 201212062), and was conducted in accordance with the protocol of the Declaration of Helsinki. Twelve cadaveric male human knees with an average age of 30.6 years (ranging from 25 to 38

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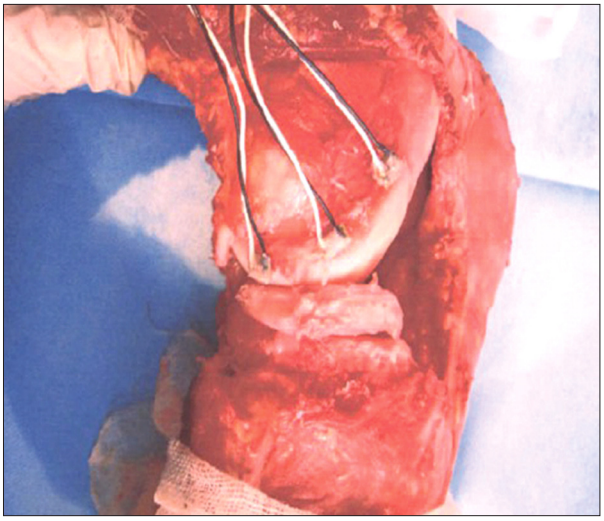

Figure 1. The installment of strain gauges at anterior, middle, and posterior part of the lateral femoral condyle.

years) were used as specimens. The causes of death were accidents and other causes that did not harm the normal structure and function of the knee. The donors' relatives were informed of the purpose of our research and signed a consent form. All of the cadaveric knees underwent macroscopic inspection and the posterior drawer test to rule out gross anomalies, degeneration, fractures, tumors, and PCL damage. Each whole knee was cut with 30 cm of both the femoral and tibial side attached and the adjacent soft tissues removed, leaving soft tissues surrounding the intact knee joint. The ends of the femur and tibia were then fixed in cylinders to enable rigid fixation during testing. The posterior articular capsule was incised to expose but not sever the PCL through a posterior midline incision.

Grouping of partial and complete PCL ruptures and test procedure

The mechanical method used was that described in our previous study [20]. Briefly, the specimens were divided into 4 groups: the intact PCL group (n=12), the ALB rupture group (n=6), the PMB rupture group (n=6), and the PCL rupture group (n=12), following the order in which experiments were conducted. All of the transections were made at the distal third of the ligament.

A lateral parapatellar incision and a lateral femoral condyle posterolateral incision were made to expose the anterior, middle, and posterior parts of the lateral femoral condyle. Three strain gauges were installed on these parts (Figure 1), and the joint capsule was closed with a suture. A static strain-measuring device was used to measure and record the strain at these sites under 200 N, 400 N, 600 N, and 800 N loads at 0°, 30°, 60°, and 90° of flexion. The specimens were then randomly divided into the ALB rupture group (n=6) in which the ALB was transected, and the PMB rupture group (n=6) in which the PMB was transected. The test procedure was repeated in these 2 groups. Finally, the PCL of all 12 specimens was completely transected to create the PCL rupture group, and the same test procedure was repeated.

Animal model of PCL rupture

The animal experiment was carried out in accordance with relevant guidelines and regulations, and was approved by the Medical Ethics Committee of Xiangya Hospital, Central South University (Grant number: 201212067). The study included 48 mature male rabbits (2.5±0.4 kg, 6 months of age), raised in the Animal Center of Central South University. The surgical procedure was carried out in accordance with our previous study [17]. Briefly, after anesthesia, a medial patellar incision was made to dissect the joint capsule, then expose and transect the PCL in the flexion position of the knee. The incision was closed without fixation of the knee joint. The same surgery was carried out on the contralateral side without PCL transection.

Morphology

At weeks 4, 8, 16, and 24 after surgery, 12 rabbits were euthanized by air embolism. The PCL rupture models were validated using the posterior drawer test. Both knees were harvested and the morphological characteristics of the lateral femoral condyle were observed, including surface flatness, color, flexibility, and intactness.

Histology

The histological method used was that described in our previous study [17]. Briefly, the paraffin section of the medial tibial plateau was serially sliced and HE and immunohistochemical staining were performed. The sections were incubated with 1: 200 rabbit polyclonal antibody MMP-7 or collagen type II overnight, then incubated with rabbit immunoglobulin G. Light microscopy was used to evaluate the histological changes of the medial meniscus sections that were quantified with an appropriate scoring system [21,22]. The Motic Images System was used to evaluate the expression intensity of MMP-7 or collagen type II in the specimens. The area of the specimens was used for cell number correction by light microscopy (at least 10 non-overlapping fields per side of 1 rabbit) and the results are shown as the positive cell rate (positively stained cell number/total cell number ×100%).

Bone densitometry

The BMD of the lateral femoral condyle of both sides of the knees was measured by dual-energy x-ray absorptiometry

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(Hologic QDR-4500A) equipped with the appropriate software for bone assessment in small animals [23]. The scan resolution was 1.0×1.0 mm with scan speed of 10 mm/s. The coefficient of variation for repeated measurements on the same bone was <1.0%.

Statistical analysis

SPSS (version 16.0 for Windows; SPSS Inc., Chicago, IL) was used for the data management and statistical analysis. Data are expressed as the mean ±standard deviation. The paired *t* test was used to evaluate paired data, and the SNK-q test (Student-Newman-Keuls test) was used to evaluate pairwise comparisons, on condition that the mean of the data met criteria for homogeneity of variance, while the Dunnett's-T3 test was used when the mean of the data did not meet homogeneity of variance. For non-parametric tests, the Nemenyi rank-sum test and Wilcoxon rank-sum test were used. Differences with P<0.05 were taken to be statistically significant.

Results

Strain on the anterior, middle, and posterior parts of the lateral femoral condyle under various loads at the 0° position

The strain on the middle part of the lateral femoral condyle was negative (pressing strains), while that on the anterior and posterior parts was positive (tensile strains) in all groups under various loading conditions at 0° of knee flexion (Table 1). Under a 200 N loading force, the strain on all parts did not differ significantly between the 4 groups (P>0.05). Under 400 N and 600 N loading forces, the strain on all parts did not differ significantly between the intact PCL group and the ALB rupture group or between the PMB rupture group and the PCL rupture group (P>0.05), while the absolute values of strain on all parts in the intact PCL group and the ALB rupture group were significantly smaller than those in the PMB rupture group and the PCL rupture group (P<0.05). Under an 800 N loading force, the difference between the strain in all of the groups on all parts was significant (P<0.05). The correlation of the absolute value of strain in each group on all parts was: the PCL rupture group > the PMB rupture group > the ALB rupture group > the intact PCL group.

Strain on the anterior, middle, and posterior parts of the lateral femoral condyle under various loads at 30° flexion

When flexed to 30°, under 200 N, 400 N, and 600 N loading forces, the strain on the anterior and posterior parts was a pressing strain in all groups (Table 1). The strain on all parts did not differ significantly between the intact PCL group and the PMB rupture group or between the ALB rupture group and the PCL rupture group (both P>0.05), while the absolute values of strain on all parts in the intact PCL group and the PMB rupture group were significantly smaller than those in the ALB rupture group and the PCL rupture group (all P<0.05). The strain on the middle part was tensile strain in the intact PCL group and the PMB rupture group with no significant difference between the 2 groups (P>0.05), but was compression strain in the ALB rupture group and the PCL rupture group, with no significant difference between the 2 groups (P>0.05). Under an 800 N loading force, the strain on the anterior part was compression strain in all groups, with significant differences between the intact PCL group and the PMB rupture group and the ALB rupture group and the PCL rupture group (P<0.05). The correlation of the absolute value of strain in each group was: the PCL rupture group > the ALB rupture group > the intact PCL group > the PMB rupture group. The strain on the middle part was tensile strain in the intact PCL group and the PMB rupture group, but was compression strain in the ALB rupture group and the PCL rupture group, with significant differences between the groups (P<0.05). The correlation of the absolute value of strain was: the PMB rupture group > the intact PCL group, the PCL rupture group > the ALB rupture group. The strain on the posterior part was compression strain in all groups, with significant differences between the intact PCL group and the PMB rupture group and between the ALB rupture group and the PCL rupture group (P<0.05). The correlation of the absolute value of strain on all parts in each group was: the PCL rupture group > the ALB rupture group > the PMB rupture group > the intact PCL group.

Strain on the anterior, middle, and posterior parts of the lateral femoral condyle under various loads at 60° flexion

At the 60° position, under 200 N, 400 N, and 600 N loading forces, the strain on all parts was compression strain in all groups (Table 1). No significant difference in strain was observed between the intact PCL group and the PMB rupture group or between the ALB rupture group and the PCL rupture group (both P>0.05), while the absolute values of strain of the former were significantly smaller than those of the latter (P<0.05). Under an 800 N loading force, the strain on all parts was compression strain in all groups, with significant differences between the intact PCL group and the PMB rupture group and between the ALB rupture group and the PCL rupture group (both P<0.05). The correlation of the absolute value of strain on the anterior part in each group was: the intact PCL group > the PMB rupture group > the PCL rupture group > the ALB rupture group; while that on the middle and posterior parts was: the PCL rupture group > the ALB rupture group > the PMB rupture group > the intact PCL group.

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Strain on the anterior, middle, and posterior parts of the lateral femoral condyle under various loads at 90° flexion

The strain on all parts was compression strain in all groups under various loading conditions at 90° of flexion, with significant differences between the intact PCL group and the PMB rupture group and between the ALB rupture group and the PCL rupture group (both P<0.05; Table 1). The correlation of the absolute value of strain on the anterior part in each group was: the intact PCL group > the PMB rupture group > the PCL rupture group > the ALB rupture group; while that on the middle and posterior parts was: the PCL rupture group > the ALB rupture group > the PMB rupture group > the intact PCL group.

Morphology and histology of the lateral femoral condyle in the rabbit model of PCL rupture

Compared with the control groups, the cartilage of the lateral femoral condyle in the PCL rupture group presented noticeable degenerative characteristics (Table 2). The HE and immunohistochemical staining of the lateral femoral condyle in the PCL rupture groups showed time-dependent abnormities and deterioration in comparison with the control groups, indicating that PCL rupture may act as a progressive degenerative factor of the lateral femoral condyle (Figure 2).

Table 1. Strain in the anterior, middle, and posterior pats of the lateral femoral condyle in all groups under various loading conditions at different flexion angles ($\bar{\chi}$±s, με).

Flexion angles	Parts	Groups	200 N	400 N	600 N	800 N
0°	Anterior	PCL intact group	24.72±2.30	34.42±1.93	44.37±1.85	49.64±2.28
		ALB rupture group	26.17±1.41*	34.53±1.21	45.43±1.41	52.17±1.72[a]
		PMB rupture group	27.63±1.63*	39.67±1.87[a,b]	47.21±1.86[a,b]	57.87±1.47[a,b]
		PCL rupture group	28.23±3.56*	40.55±2.41[a,b]	49.64±1.85[a,b]	61.17±2.44[a,b,c]
	Middle	PCL intact group	–30.67±2.27	–41.58±3.17	–51.83±2.52	–59.17±2.76
		ALB rupture group	–31.17±2.32	–41.67±2.16	–53.00±1.79	–64.00±3.41[a]
		PMB rupture group	–32.50±2.74	–47.67±2.80[a,b]	–59.00±2.83[a,b]	–69.33±1.75[a,b]
		PCL rupture group	–33.83±2.51	–47.92±3.12[a,b]	–59.92±3.12[a,b]	–72.92±2.07[a,b,c]
	Posterior	PCL intact group	18.91±3.45	22.33±3.42	28.00±3.16	28.58±2.27
		ALB rupture group	20.17±2.78	22.50±4.23	29.83±2.14	31.50±1.87[a]
		PMB rupture group	20.83±2.86	26.83±2.99[a,b]	32.67±2.87[a,b]	34.50±1.87[a,b]
		PCL rupture group	21.67±3.57	27.50±2.78[a,b]	33.51±2.42[a,b]	38.50±2.31[a,b,c]
30°	Anterior	PCL intact group	–12.58±1.56	–17.91±2.81	–19.50±3.50	–25.25±2.30
		ALB rupture group	–13.50±1.05[a]*	–17.83±1.57[a]	–23.67±2.42[a]	–28.67±3.01[a]
		PMB rupture group	–11.50±2.56[b]*	–16.73±2.16[b]	–18.00±2.10[b]	–21.83±2.79[a,b]
		PCL rupture group	–17.50±2.29[a,c]*	–22.15±2.30[a,c]	–27.25±2.30[a,c]	–31.83±3.74[a,b,c]
	Middle	PCL intact group	50.33±2.47	57.33±2.46	62.17±2.46	69.58±2.07
		ALB rupture group	–55.83±0.75[a]	–65.50±1.87[a]	55.50±2.07[a]	–67.08±2.43[a]
		PMB rupture group	49.33±3.01[b]	55.50±2.07[b]	63.83±1.47[b]	72.33±2.16[a,b]
		PCL rupture group	–58.33±1.97[a,c]	–67.08±2.43[a,c]	–69.33±1.97[a,c]	–77.92±2.07[a,b,c]
	Posterior	PCL intact group	–12.33±2.58	–12.50±2.17	–13.83±2.79	–15.83±2.48
		ALB rupture group	–28.50±2.88[a]	–34.17±1.47[a]	–38.50±3.08[a]	–40.67±1.86[a]
		PMB rupture group	–12.50±1.62[b]	–13.67±2.57[b]	–14.42±3.15[b]	–22.75±2.56[a,b]
		PCL rupture group	–28.83±3.32[a,c]	–34.58±2.35[a,c]	–40.50±2.47[a,c]	–49.83±3.51[a,b,c]

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Table 1 continued. Strain in the anterior, middle, and posterior pats of the lateral femoral condyle in all groups under various loading conditions at different flexion angles ($\bar{\chi}\pm s$, με).

Flexion angles	Parts	Groups	200 N	400 N	600 N	800 N
60°	Anterior	PCL intact group	−14.67±2.53	−24.17±3.46	−33.67±3.60	−44.75±2.80
		ALB rupture group	−15.17±1.47[a]	−22.17±1.47[a]*	−24.50±2.43[a]	−31.17±2.64[a]
		PMB rupture group	−14.67±2.16[b]	−21.83±2.79[b]*	−31.67±1.75[b]	−40.33±3.01[a,b]
		PCL rupture group	−14.67±2.53[a]	−24.83±4.59[a]*	−27.75±3.62[a]	−37.67±4.52[a,b,c]
	Middle	PCL intact group	−25.08±2.47	−33.25±2.34	−42.67±1.50	−48.00±3.13
		ALB rupture group	−29.33±2.16[a]	−35.67±1.63[a]	−49.17±1.47[a]	−55.67±1.50[a]
		PMB rupture group	−26.17±1.47[b]	−32.00±1.78[b]	−42.83±2.32[b]	−52.17±1.48[a,b]
		PCL rupture group	−31.33±2.35[a,c]	−37.25±2.34[a,c]	−51.58±1.56[a,c]	−61.83±2.82[a,b,c]
	Posterior	PCL intact group	−12.67±1.21	−18.50±2.43	−21.00±2.37	−29.33±1.86
		ALB rupture group	−32.33±1.37[a]	−36.83±2.14[a]	−45.67±2.07[a]*	−45.67±3.77[a]
		PMB rupture group	−13.58±2.15[b]	−19.92±4.23[b]	−23.58±2.15[b]*	−33.67±3.05[a,b]
		PCL rupture group	−33.75±2.56[a,c]	−38.83±2.51[a,c]	−48.67±3.25[a,c]*	−51.75±2.56[a,b,c]
90°	Anterior	PCL intact group	−26.33±3.67	−38.42±4.50	−48.33±3.03	−56.17±5.02
		ALB rupture group	−20.00±4.65[a]	−23.67±2.88[a]	−28.50±2.07[a]	−33.83±2.79[a]*
		PMB rupture group	−19.67±2.16[a,b]	−34.33±2.16[a,b]	−42.83±1.47[a,b]	−52.17±3.19[a,b]*
		PCL rupture group	−26.25±4.29[a,b,c]	−28.75±4.41[a,b,c]	−31.92±3.55[a,b,c]	−39.25±2.45[a,b,c]*
	Middle	PCL intact group	−23.17±2.76	−28.67±2.57	−32.33±1.50	−36.50±2.65
		ALB rupture group	−29.50±1.87[a]	−37.50±1.87[a]	−42.50±3.02[a]	−47.67±2.16[a]
		PMB rupture group	−25.83±1.47[a,b]	−32.17±1.47[a,b]	−38.17±2.32[a,b]	−43.33±1.51[a,b]
		PCL rupture group	−33.50±2.32[a,b,c]	−40.3±2.42[a,b,c]	−47.75±1.48[a,b,c]	−54.92±2.39[a,b,c]
	Posterior	PCL intact group	−12.50±1.87	−20.83±1.47	−22.50±2.67	−29.00±2.19
		ALB rupture group	−26.33±1.97[a]	−38.33±2.06[a]*	−40.67±3.88[a]	−48.33±4.46[a]
		PMB rupture group	−19.83±4.24[a,b]	−25.83±4.23[a,b]*	−29.41±5.12[a,b]	−34.41±2.61[a,b]
		PCL rupture group	−39.41±2.47[a,b,c]	−43.33±2.99[a,b,c]*	−46.96±5.03[a,b,c]	−57.67±2.31[a,b,c]

με – micro-strain; PCL – posterior cruciate ligament; ALB – anterolateral band; PMB – posteromedial band.[a] P<0.05 compared with PCL intact; [b] P<0.05 compared with ALB rupture; [c] P<0.05 compared with PMB rupture; * Dunnett T3 test, others SNK-q test.

Table 2. Morphological characteristics of lateral femoral condyle between control group and PCL rupture group.

	Control group	PCL rupture group			
	All time points	4th week	8th week	16th week	24th week
Structural integrity	Integrated	Integrated	Integrated	Worn free edge	Ulcer
Surface	Smooth	Smooth	Not smooth	Rough	Rough
Color	Bright white	Light blue	Faint yellow	Grey-yellow	Yellow
Elasticity	Good	Good	Slight slack	Slack	Slack

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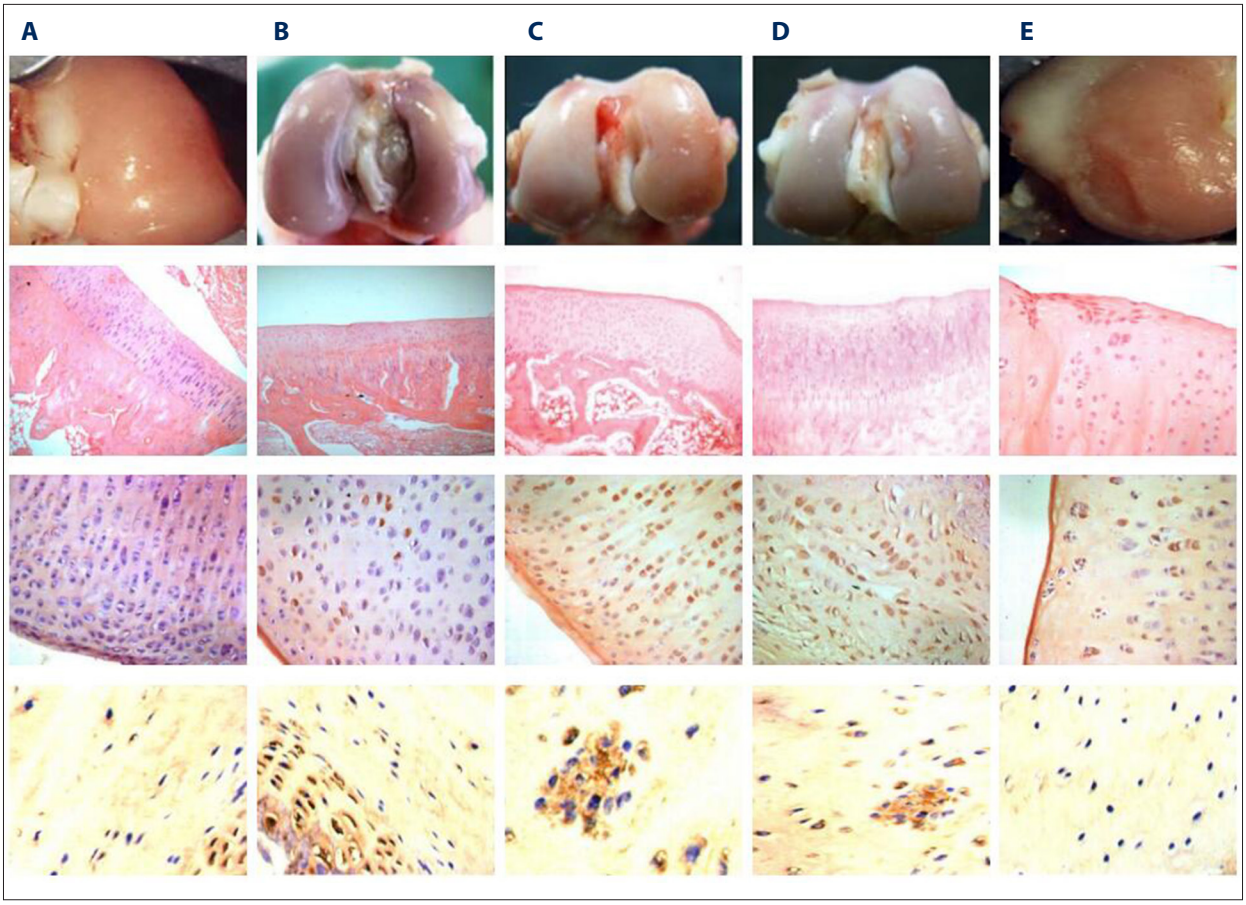

Figure 2. The images were visualized with macro-observation (**first row**), HE staining (**second row**), MMP-7 immunohistochemical staining (**third row**), and collagen type II immunohistochemical staining (**fourth row**) in PCL rupture side and the control side of the lateral femoral condyle. (**A**) the control side: continuous and smooth surface, chondrocytes in columnar shape and regularly ranged, even nuclear size and staining, mild deep staining in cytoplasm, continuous tidemark; weakly MMP-7 positive-staining expressed in superficial layer, few in cytoplasm; even collagen type II expression in ECM, few positive-staining cells, and almost no staining in cytoplasm; (**B**) 4th week after PCL rupture: smooth and approximately flat surface, chondrocytes regularly arranged, clear tissue layers, even HE staining, and continuous tidemark; MMP-7 positive-staining weakly expressed in surface, matrix, and cytoplasm, full oval-shaped nuclear, partly expressed in cytoplasm; uneven collagen type II positive-staining in matrix, partly expressed in cytoplasm; (**C**) 8th week after PCL rupture: slight rough surface, chondrocytes arranged disorderly, few gathered in clusters, unclear layers, uneven staining; MMP-7 strongly expressed in superficial layer, positive in matrix and cytoplasm, deeper than 4th week; more collagen type II positive-staining cells; (**D**) 16th week after PCL rupture: rough surface, chondrocytes arranged disorderly, more clusters, disorderly arrangement tissue layers; MMP-7 strongly expressed in superficial layer, matrix and cytoplasm, matrix partly degraded, cytomorphosis; more collagen type II positive-staining cells; (**E**) 24th week after PCL rupture: superficial layer fibrosis, cell number decreased obviously, layers disorderly arranged; decreased MMP-7 staining, especially in cytoplasm, matrix degradation, uneven cell size and shape; few collagen type II positive-staining cells.

Mankin score, BMD, and the MMP-7 and collagen type II expression levels of cartilage damage in the lateral femoral condyle after PCL rupture

Makin scores, BMD, and the MMP-7 and type II collagen expression level in the cartilage of the lateral femoral condyle in control and PCL rupture groups at all time points are presented in Table 3, and a pairwise comparison between all of the time points is presented in Table 4. The Mankin score of the lateral femoral condyle cartilage in the PCL rupture groups was significantly higher than that in the control group at all time points (all $P<0.05$). In the PCL rupture groups, the Mankin scores increased continuously from week 4 to week 24 after surgery and the difference was significant ($P<0.05$), while little change was observed in the control group ($P>0.05$).

No significant difference in BMD values was found between the PCL rupture group and the control group at any time point

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Table 3. Comparison of Mankin scores ($\bar{\chi}$±s), BMD($\bar{\chi}$±s), MMP-7 ($\bar{\chi}$±s, %) and collagen type II expression rate ($\bar{\chi}$±s, %) in lateral femoral condyle at all time points between PCL rupture group and control group (Wilcoxon rank-sum test).

		4th week	8th week	16th week	24th week
Mankin Score	PCL rupture group	1.42±0.52	3.59±1.16	6.08±1.73	8.25±0.62
	Control group	0.62±0.52	0.60±0.52	0.67±0.65	0.75±0.62
	P value	<0.05	<0.05	<0.05	<0.05
BMD	PCL rupture group	0.25±0.03	0.31±0.06	0.32±0.04	0.40±0.08
	Control group	0.30±0.12	0.31±0.07	0.32±0.13	0.35±0.05
	P value	>0.05	>0.05	>0.05	>0.05
MMP-7	PCL rupture group	9.18±1.11	9.50±1.35	9.50±1.41	10.19±1.77
	Control group	15.89±1.42	35.18±4.22	51.08±3.59	51.93±3.21
	P value	<0.05	<0.05	<0.05	<0.05
Collagen type II	PCL rupture group	15.58±1.37	15.92±1.61	14.81±1.44	14.88±1.67
	Control group	20.95±2.19	25.38±2.46	14.75±2.17	10.19±1.45
	P value	<0.05	<0.05	>0.05	<0.05

BMD – bone mineral density; PCL – posterior cruciate ligament. Continuous data are expressed as the mean ± standard deviation. P value of less than 0.05 were considered to be statistically different.

Table 4. Pairwise comparison of Mankin scores, BMD, MMP-7, and collagen type II expression rate in lateral femoral condyle at various time points between PCL rupture group and control group (P value).

		4 W: 8 W	4 W: 16 W	4 W: 24 W	8 W: 16 W	8 W: 24 W	16 W: 24 W
Mankin score	PCL rupture group	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
	Control group	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05
BMD	PCL rupture group	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05
	Control group	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05
MMP-7	PCL rupture group	<0.05	<0.05	<0.05	<0.05	<0.05	>0.05
	Control group	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05
Collagen type II	PCL rupture group	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
	Control group	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05

BMD – bone mineral density; PCL – posterior cruciate ligament. P values of less than 0.05 were considered to show statistically significant differences. Dunnett T3 test, others Nemenyi rank test.

(P>0.05), and BMD of both groups remained relatively stable during the whole procedure (P>0.05).

All of the PCL rupture groups had higher expression levels of MMP-7 and collagen type II than the control groups at all time points (P<0.05) (Figure 2). The pairwise comparison of MMP-7 expression among all of the time points showed higher MMP-7 expression at the later than at the earlier time points except for the comparison between weeks 16 and 24 (Table 4), while collagen type II expression increased continuously from week 4 to week 24 and the difference was significant (P<0.05). Both MMP-7 and type II collagen expression levels showed little change in the control group throughout the procedure.

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Discussion

The present study was conducted to investigate BMD and the biomechanical and histological effects of PCL rupture on the lateral femoral condyle. Based on the experimental results of human and rabbit specimens, our study suggests that PCL rupture may trigger a coordinated response of lateral femoral condyle degeneration in a time-dependent manner, largely due to the high level of expression of MMP-7 and collagen type II.

Normal articular cartilage is generally affected by the pressure from body weight and muscle contraction, mainly manifested as the compressive stress in the vertical direction, and the tensile stress that leads to the cartilage deformation parallel to the articular surface. Pressure stress may transform into tensile stress when the articular cartilage is loaded; local high stress and correlated cartilage damage may therefore be avoided. However, when the stress state of the knee joint is not the same as the normal condition, the articular cartilage of the knee changes accordingly. During the experiment, a static strain-measuring device was used to measure and record the strain at the anterior, middle, and posterior parts of the lateral femoral condyle under 200 N, 400 N, 600 N, and 800 N loads at 0°, 30°, 60°, and 90° of flexion. The human knee bears 85.6% of the total weight at the standing point, and a single knee bears loads of at least 200 N by weight conversion [24,25]. Therefore, a 200 N axial load was chosen as the lower limit. The weight of the load on the knee during movement is 2–3 times greater [26]. The maximum load on the PCL should be less than the ultimate stress during daily activity; therefore, we chose 800 N, which is less than twice the weight, was chosen as the upper limit load to avoid specimen damage, simulating long-term load change-induced cartilage degeneration and OA. We selected 4 angles to simulate normal knee flexion and extension during daily activities: 0° to represent the standing position, and 30°, 60°, and 90° to represent normal walking swing, maximum walking flexion, and swift running angles, respectively. Greater flexion angles were ignored to avoid specimen damage.

Clinically, partial PCL ruptures are more typical and the corresponding treatment decision is also controversial. Even if the PCL is partially broken, it may also damage the local biomechanical environment of the articular cartilage, which may lead to chronic damage. To establish a theoretical basis for the clinical treatment of partial PCL ruptures, we studied the effect of partial PCL ruptures on the biomechanical properties of the lateral femoral condyle. In the human tissue anatomy experiment, our results indicated that, although isolated ALB or PMB bundle ruptures did not cause severe instability of the knee, redistribution of the strain changed the biomechanics of the lateral femoral condyle. At the 0° position, the PMB played the major role in maintaining joint stability under loads of less than 600 N, while under greater loads the ALB joined in maintaining joint stability to some extent. During flexion from 30° to 60°, the ALB played the major role in maintaining joint backward stability under loads of less than 600 N. The PMB also acts in maintaining joint stability to some extent under greater loads. At 90° of flexion, both the ALB and PMB maintained knee stability, while the ALB played the major role and the PMB a secondary role, which is in accordance with previous study outcomes, and provides solid theoretical support for the classification of the PCL bundle into the ALB and PMB [2]. We also found that, in addition to the PCL, other structures in the knee also maintain its backward stability under low loads at the extension position, which provides feasible theoretical support for the conservative treatment of a single PCL injury, especially a partial PCL rupture.

Furthermore, we established a rabbit experimental model to verify the results from the human experiment and to further explore the biomechanical and histological effects of PCL rupture on the lateral femoral condyle. Selection of an animal model similar to the bone structure and pathogenesis of OA in humans is an ideal tool for the study of altered bone structure and OA. Previous studies have investigated the relationship between cartilage injury and OA with the establishment of experimental animal models in rabbits or rats [27–29]. In the present study, the design of the animal study was mainly based on previous research on articular cartilage degeneration secondary to PCL rupture in rabbit knees [30]. The ligament was exposed and transected using a patellar medial incision, and mild, moderate, and severe articular cartilage damage were observed at 6, 12, and 24 weeks after PCL rupture [30]. For closer and more accurate observations, we chose 4, 8, 16, and 24 weeks after PCL rupture as time points to monitor lateral femoral condyle changes.

Our study used the dual-energy x-ray absorptiometry method to measure changes in the BMD of the whole lateral femoral condyle to eliminate the confounding factor of man-made division. Etiological study has suggested that OA is a result of degradation of and synthesis imbalance among the chondrocyte, extracellular matrix, and subchondral bone caused by multiple mechanical and biological factors [31]. Previous studies proved that the BMD of subchondral bone increased in OA, and was correlated with OA pathogenesis [32,33]. Therefore, the measurement of BMD can reflect the biomechanical changes of PCL rupture-induced OA. The BMD was generally considered to be elevated at the end stage of OA, with symptoms of limping and joint movement limitation [34,35]. In our study, although not significantly different from the control group, BMD was mildly decreased but not elevated 4 weeks after surgery. We supposed that no obvious cartilage degeneration or endochondral ossification had occurred in the lateral femoral condyle because elevated BMD is a feature of late-stage OA. Another

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possibility is that the rabbits had reduced lower limb activities because of the pain caused by the surgery, and disuse-osteoporosis caused the decreased BMD. Furthermore, BMD in the experimental group was elevated at 24 weeks when compared with the control group and other time points, but the difference was not statistically significant. According to the study conducted by Atalar, although a main characteristic of late-stage OA is osteophyte formation, the BMD is not affected [36]. Similarly, our study also proved that BMD was not elevated, although noticeable degradation was observed at the later time points after PCL rupture. Another possibility for our drawing the opposite conclusion was the limited number of participants and short observation time, which still needs further investigation.

Of great importance, activities of MMP-7 and collagen type II in articular cartilage were also investigated to illustrated the relationship of those 2 proteins with PCL rupture and the progression of OA. In normal articular cartilage, the main components of the extracellular matrix are collagen type II fibrils and proteoglycans, which are distinctively distributed in various anatomical zones of the cartilage [37]. The direct cause of the loss of normal biomechanical properties of the articular cartilage is the change of collagen type II and proteoglycans, and their changes have been suggested to be closely correlated with the progression of OA. Vikkula found that early-onset OA was linked to the type II procollagen gene (COL2A1) [38]. We observed even immunohistochemical staining of collagen type II in the extracellular matrix, and no staining in the chondrocyte in the lateral femoral condyle of normal knees. After PCL rupture, immunohistochemical staining became deeper in the extracellular matrix, indicating increased collagen content. The staining intensity of intracellular collagen staining was also deeper, indicating the enhanced collagen synthesis ability of chondrocytes. Therefore, under long-term abnormal strain, the chondrocyte in the lateral femoral condyle was stimulated to initiate cartilage degradation, and more extracellular matrix-like collagen was secreted to adapt to the changes in strain.

MMP-7, also known as matrilysin, is a unique member of the MMP family. It has a specific ability to degrade various extracellular matrix components such as cartilage proteoglycan, type II collagen, gelatin, fibronectin, and laminin [39]. Moreover, MMP-7 can also enhance the degradation of the extracellular matrix by activating other MMPs, such as MMP-2 and MMP-9 [40]. In accordance with a previous study [41], MMP-7 was mainly expressed in the cytoplasm of chondrocytes, and barely or weakly expressed in normal chondrocytes, but was highly expressed in degraded cartilage. Previous evidence has shown that OA may appear under the situation of imbalanced TIMP and MMP levels in the articular cartilage and synovial fluid; the lack of TIMP causes the increase of MMP activity thereby exerts a role in matrix degradation [42,43]. The immunohistochemical staining current results in the present study also showed little MMP-7 expression in normal cartilage, but high expression in the cartilage of the lateral femoral condyle in the PCL rupture groups, which was significantly enhanced over time, indicating that MMP-7 might play important roles in the pathogenesis of OA.

Conclusions

PCL rupture can cause abnormal loads on all parts of the lateral femoral condyle with any axial loading and at all positions. Noticeable time-dependent degenerative changes in the lateral femoral condyle after PCL rupture were observed histologically. The decreased expression of MMP-7 and collagen type II in articular cartilage may be responsible for the degeneration, and PCL rupture may be the trigger of lateral femoral condyle degradation and, ultimately, OA.

Conflicts of interest

The authors report no conflicts of interest in this work.

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