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A *Macaca Fascicularis* Knee Osteoarthritis Model Developed by Modified Hulth Combined with Joint Scratches

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Background: Osteoarthritis is a common degenerative disease of joints, and animal models have important significance in the investigation of this disease. The aim of this study was to develop a better method for developing osteoarthritis models in primates by comparing the modified Hulth score combined with joint scratches modeling method with others.

Material/Methods: We randomly divided 15 young male *Macaca fascicularis* and 3 old male *Macaca fascicularis* into 6 groups (n=3). Knee osteoarthritis (KOA) models were developed with different methods: modified Hulth combined with joint scratches (Group A), modified Hulth (Group B), Hulth (Group C), spontaneous models (Group D); sham-operated (Group E), and blank control (Group F). Morphology and pathology of knee joints were observed at the 8th week after surgery. The levels of WBC, IL-1β, and TGF-β1 in synovial fluid were detected by ELISA. The levels of COL-II, ACAN, and MMP-13 in articular cartilage were examined by RT-qPCR and Western blot.

Results: In Brittberg and modified Mankin score, Group A was higher than B ($P<0.05$) and lower than C ($P<0.05$), and there was no statistically significant difference between Group A and D ($P>0.05$). Except for Group E and F, the differences were statistically significant among others in WBC, IL-1β, and TGF-β1 ($P<0.05$). COL-II and ACAN decreased and MMP-13 increased, and there was no significant difference between Groups A and D ($P>0.05$) or between Groups E and F ($P>0.05$). There were statistically significant differences among other groups ($P<0.05$).

Conclusions: The models developed by modified Hulth combined with joint scratches were the closet to spontaneous models at the 8th week after surgery.

MeSH Keywords: **Cartilage • *Macaca Fascicularis* • Models, Animal • Osteoarthritis**

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Background

Osteoarthritis (OA) is a common degenerative joint disease, and knee osteoarthritis (KOA) is the most common [1]. KOA is caused by factors such as age, obesity, strain, trauma, congenital abnormality, and deformity. KOA is manifested as degeneration of articular cartilage and hyperplasia of articular borders and subchondral bone. The pathological changes often involve articular cartilage, subchondral bone, synovium and other tissues, in which the articular cartilage is the most commonly involved [2,3]. The main symptoms are swelling of the joints, pain, and progressive dysfunction. KOA has become a major cause of disability [4,5] and causes serious socio-economic burdens worldwide [6]. By 2030, 25% of the population in the United States may suffer from KOA [7], which would produce a social economic burden of $100–200 billion USD [8,9]. The number of KOA patients in developing countries may increase in the future because of the aging population, lifestyle, and the relative lack of medical resources.

The pathogenesis of OA has become a focus of research, but the specific mechanism is still not clear [10]. Animal models, as an important tool to study the pathogenesis of KOA, have attracted much attention. Developing a reasonable animal model is of great significance to exploring the pathogenesis and treatment of KOA [11,12]. Over the past half century, researchers have developed various KOA models through surgical and non-surgical methods. In addition, there are also spontaneous models. The commonly used experimental animals are mice, rats, guinea pigs, rabbits, dogs, pigs, goats, sheep, horses, and primates. McCoy et al. [11] made a statistical classification of the experimental animals that have been used in the past through a literature review, and found that rats were the most used small animals (77%), and dogs were the most used large animals (23%). *Macaca fascicularis* and other primates are similar to humans in structure, physiology, and behavior, and closely resemble changes in human disease. However, there had been little research using KOA models such as *Macaca fascicularis* or other primates.

Surgical modeling has been widely used, including complete or partial meniscectomy, meniscus injury, ACL and PCL transection, medial or lateral collateral ligament transaction, osteotomy, joint cavity implanting bone fragments, and joint scratches [11,12]. The mechanical balance of joints is disturbed by one or the combination of more methods, which can result in cartilage lesions. These methods are rapid and reproducible and they were widely used in the development of KOA model. However, due to a lack of research comparing these various methods, it is still unclear how to choose a fast, convenient, and less-traumatic modeling method.

In this study, a horizontal comparison was made among KOA models of *Macaca fascicularis*. These models were developed by the modified Hulth method combined with joint scratches, modified Hulth, Hulth method, and spontaneous models. The aim of this study was to determine a suitable method for modeling primate KOA. The feasibility and validity of the method were also analyzed through model comparison.

Material and Methods

Ethics statement

All experimental procedures were reviewed by the Yunnan Yingmao Biotechnology Co. Ltd. Experimental Animal Ethics Committee (No. 2016001). Animal care followed the Guidelines for Use and Care of Laboratory Animals (Office of Science and Health Reports CPRR/NIH 1996).

Animals

We obtained 15 SPF young male *Macaca fascicularis* (3–5 years old, weight 5–6.7 kg) and 3 SPF old *Macaca fascicularis* (23–25 years old, weight 5.6–6.8 kg) from Yunnan Yingmao Biotechnology Co., Ltd., with accreditation for animal research facilities by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALACI).

All *Macaca fascicularis* were cage-reared (1.5×2 m) in an SPF-level animal room at Yunnan Yingmao Biotechnology, Ltd., Animal Breeding Center after the operation, with free rest and eating. Animals were kept in an environment with 12h-12h light: dark cycle, temperature 22–24°C, and relative humidity 45–55%. All animals were fed daily and had free access to water. They were allowed to move freely for 6–8 h each day in a spacious activity room (12.5×8×4 m) equipped with small rockery, swings, and ball games. Music, video and other entertainment were occasionally provided for relaxation.

Grouping and modeling

We randomly divided the 15 young male *Macaca fascicularis* into 5 groups by digital method, and there were 3 in each group. The 3 old male *Macaca fascicularis* were selected randomly as the spontaneous group. The ACLs of *Macaca fascicularis* were cut and the knee joint femoral condyles were scratched in Group A (n=3); the ACLs of animals were transected alone in Group B (n=3); ACLs and PCLs, medial collateral ligaments, and medial meniscus were cut in Group C (n=3); *Macaca fascicularis* in Group D (n=3) were spontaneous models; Group E (n=3) was the sham-operated group and we only exposed the joint cavity, without destroying any structure of the joint, and Group F (n=3) was the blank control group.

The animals were denied food and water for 8 h before the operation, and were fixed in supine position on the operating table

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after being anesthetized with Zoletil 50 (France Vic, 5 mg/kg, intramuscular injection) and applying a tourniquet to the proximal left leg. Then, the skin was prepared, povidone was used for disinfection, and sterile treatment towels were spread.

Firstly, a 4-cm incision was made in the median knee, and cartilage, ACL, meniscus and other structures were determined to all be in good condition in operative exploration. Secondly, ACLs of animals in Group A were cut and the knee joint femoral condyles were scratched; we cut the ACLs in Group B; we cut the cruciate ligament, medial collateral ligament, and medial meniscus in Group C; *Macaca fascicularis* of group D were spontaneous models; and group E was the sham-operated group in which we only opened the joint cavity without destroying joint structure. Group F was the blank control group. Next, articular cavity and incision were flushed with physiological saline and sutured with 3-0 absorbable suture (Alcon, Alcon Pharmaceutical Co. Ltd., USA Alcon, Alcon Laboratories, Inc, USA). The contralateral knee was placed in the extension position with plaster after the operation. Levofloxacin hydrochloride (constant Austrian, 8 mg/kg, 1 time/12 h, IV, Heng Ao, China) and sodium chloride (100 ml) was intravenously injected to prevent infection within 3 days after the operation. Tramadol hydrochloride (Chimeter, 8 mg/kg, IM, QD) was intramuscularly injected to relieve pain. The wound healed within 14 days after the operation.

Morphological observation

Euthanasia was performed on animals under intensive anesthesia at 8 weeks after the operation. We cut the skin along the original surgical incision layer by layer in Groups A, B, C, and E, and median incision of the knee joint was made in Groups D and F. The joint cavity was exposed to collect the effusion. The gross morphology of articular cartilage was observed and graded using the Brittberg grading standard [13]. Articular cartilage of the femur and tibial plateau were cut and placed on a sterile operating table. Specimens of cartilage were cut (0.5×0.3×0.2 cm) and divided into 2 parts. One part was immediately put into 1.4% frozen paraformaldehyde (Biomics Laibo, Beijing, China) after rinsing with saline and fixed 12–24 h at 4°C, and the bone fragments were fully rinsed in 0.2 mol/L phosphate buffer (BIO, Germany) after fixation. The other one was rinsed with sterile saline at 0°C and moved into a frozen tube, temporarily saved in liquid nitrogen, and transferred to a –80°C freezer (Haier, China).

HE staining

Articular cartilage was routinely decalcified with EDTA (MERCK, Germany) and then paraffin-embedded (KUNHUA, China) and sliced into 5-μm sections. Neutral gum sealing after conventional HE staining and light microscopy (Nikon, Japan) was performed to observe tissues and slices. Each of the articular cartilage specimens was selected with 5 slices, and the modified Mankin score (Table 1) was used to evaluate the cartilage destruction [12,14,15].

ELISA

At the 8th week after modeling, the synovial fluid was collected and divided into 2 parts. One part was diluted with leukocyte dilution for 5 h and the WBC count was performed with standard cytometry. The other part was frozen at –70°C and prepared for testing. Thawed specimens were centrifuged for 15 min at 1500 rpm and supernatant was collected. Samples were diluted and added according to enzyme-linked immunosorbent assay (ELISA) kit (SignaGen, USA) operating instructions, which were incubated at 37°C for 30 min, and the concentrated solution was washed thoroughly after dilution. Then, enzymes, warm bath, washing, and color rendering were added and the termination liquid was added to terminate the reaction. IL-1β and TGF-β1 were measured within 15 min after the addition of the stop solution. The sensitivity of the IL-1β and TGF- β1 kits were 2 ng/L and 5 pg/L, respectively, and the coefficients of variation were <10% and <4.4%. Absorbance was read at 450 nm using a 2010 enzyme marker (Anthos2010, Austria), and a standard curve was made to read the contents of IL-1β and TGF-β1 in the samples.

RT-qPCR

RNA extraction and identification: 50 mg of cartilage was homogenated on ice after adding 1 ml of TRIZOL. Following the instructions of the total RNA Extraction Kit (SignaGen, USA), the concentration and purity (A260 nm/A280 nm between 1.8 and 2.0) of the extracted RNA were detected using the Nucleic Acid Protein Detector (ScanDrop, Jena, Germany) after RNA isolation. The remainder was stored in a freezer at –80°C. Primer Premier 5.0 software was used to design primers, which were synthesized by Shanghai Sangon Biological Engineering Technology & Services Co. Ltd. (Shanghai, China).
R-β-actin-F: 5'-GATCAAGATCATTGCTCCTCCTG-3', 58.93
R-β-actin-R: 5'-GTCACAGTCCGCCTAGAAGC-3', 60.46; 163 bp
R-COL-II-F: 5'-CTTCCTACGCCTGCTTTCCA-3', 60.04
R-COL-II-R: 5'-GGTGTGTTTCGTGCAGTCAT-3', 59.06; 198 bp
R-ACAN-F: 5'-ACTGGCGAGCACTGTAACAT-3', 59.68
R-ACAN-R: 5'-AGTCTTGGGCATTGTTGTTGAC-3', 59.64; 179 bp
R-MMP-13-F: 5'-TCCACTGATAACTCGCCGTC-3', 59.55
R-MMP-13-R: 5'-TCCACTGATAACTCGCCGTC-3', 60.44; 198 bp

Reverse transcription reaction: The 1-μg RNA was mixed with the first strand of the cDNA synthesis kit (SignaGen, USA) for the total extraction RNA reversed transcription to generate cDNA. The reaction system was as follows: PrimeScript RT Master Mix 2 μl, Sample corresponding to RNA 500 ng, ddH_2O 10 μl. Reaction conditions were 37°C for 15 min and 85°C for 5 s. The resulting cDNA was stored in a freezer at –20°C.

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Table 1. Modified Mankin scoring criteria.

Organization structure	Microscopic features	Scores
Cartilage structure	Normal	0
	Hierarchy is clear, arrangement is disorganize	1
	Hierarchy disorder, irregular arrangement	2
	Disorder is obvious, the hierarchy is difficult to distinguish	3
Cells	Normal cells count	0
	Mild cells hyperplasia	1
	Moderate cells hyperplasia	2
	Severe cellular hyperplasia	3
Histopatholog-ical staining	Normal	0
	Mild decrease	1
	Moderate decrease	2
	Severe decrease	3
	Uncolored	4
Tide line	Structure of tidal lines is intact	0
	Multilevel structure	1
	Tidal blur	2
	There are blood vessels through the tidal line	3

Table 2. Comparison of the basic situation of macaca fascicularis ($\bar{\chi}$±s, n=3).

Group	Age	Weight (kg)	Crown sacral length (m)
Group A	4±1	6.60±0.04	0.43±0.01
Group B	3.7±0.58	6.57±0.04	0.43±0.01
Group C	3.33±0.58	6.58±0.08	0.43±0.02
Group D	24±1*	6.67±0.05	0.46±0.01
Group E	3.68±0.58	6.57±0.05	0.42±0.01
Group F	3.33±0.58	6.60±0.07	0.44±0.02
F	374.01	1.22	2.45
P	0.001	0.36	0.09

* P<0.05 *vs.* Group D.

Table 3. Comparison of operative time and blood loss in macaca fascicularis ($\bar{\chi}$±s, n=3).

Group	Moedling time (min)	Blood loss (ml)
Group A	23.33±0.58	12.67±0.58
Group B	21.67±0.58[a]	12±1
Group C	37.67±0.58[ab]	24.33±0.58[ab]
Group E	8±1[abc]	4.33±0.58[abc]
F	883.11	408.44
P	0.001	0.001

[a] P<0.01 *vs.* Group A; [b] P<0.01 *vs.* Group B; [c] P<0.01 *vs.* Group C.

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Relative expression of each gene by RT-qPCR: SYBR Green (QIAGEN, Germany) was used as fluorescent marker and β-actin was used as internal control. We added 1 μl cDNA to 20 μl reaction system for amplification. The reaction system was: SYBR Premix Ex TaqII 10 μl, Primer F 0.3 μl, Primer R 0.3 μl, cDNA 1 μl, ddH_2O 8.4 μl. Reaction conditions were 95°C for 30 s, 95°C for 5 s, 62°C for 30 s. The reaction was cycled 40 times.

The samples were placed into the PCR instrument (Thermo, Arktik, USA) for amplification. At the end of amplification reaction, the instrument was closed and the data were analyzed. If the melting curve of each gene was observed as a sharp single peak, the specificity of the amplification sequence was strong. We independently repeated each test 3 times. The expression levels of mRNA were calculated using the $2^{-\Delta\Delta Ct}$ method after the amplification reaction.

Western blot

The proteins from frozen tissue samples were extracted using the Total Protein Extraction Kit (Besebio, Shanghai, China). Then, the protein content was measured by BCA (Pierce) method. The separating gel and stacking gel were prepared according to the kit instructions. The treated protein samples were loaded to the wells of the gel. Samples were made to run on a constant volt (80V in the stacking gel, 200V in the separating gel) until bromophenol blue (BPB) came to the bottom of the gel. The electrophoresis apparatus was disassembled, the gels were taken out, and the stacking gel was removed. The separating gel, ultra-thick filter paper, and PVDF membrane were placed in the Western blot transfer buffer for 3 min. Blotting pad, ultra-thick filter paper, separation glue, PVDF membrane, and blotting pad were placed on the side of the black panel. The blotting layers were assembled tightly and placed in the gel transfer apparatus. Western blot transfer buffer was added and the membranes were transferred at 200 mA for 2 h. The PVDF membrane was separated from the transfer apparatus and moved to a plate containing TBST solution while the transfer was finished. It was blocked on a shaker at 20°C for 1 h. Then, the membrane was incubated with primary antibodies and secondary antibodies. The PVDF membrane was soaked in the mixed liquor composed of equal volumes of ECL luminescence kit A liquid and B liquid for 5 min. Afterwards, the PVDF membrane was transferred on absorbent paper to remove the residual liquid. The chemiluminescence imager (BIORAD, Hercules, CA, USA) was used to take and save pictures.

Statistical analysis

The results are expressed as mean ± standard deviation ($\bar{\chi}\pm s$). All data were tested for normality by SPSS version 17.0 statistical software (IBM). Count data were analyzed with analysis of variance (Levene's test, one-way ANOVA, least significant difference). Nonparametric data were corrected using the Kruskal-Wallis rank sum test. The test level was $\alpha=0.05$, $P<0.05$ was viewed as a statistically significant difference.

Results

The basic situation and intraoperative condition of *Macaca fascicularis*

No infection or death occurred in any animals, and there were no significant differences in body weight and crown sacral length ($P>0.05$) (Table 2). Modeling time for Group A was longer than for Groups B, C, and E, and the differences were statistically significant ($P<0.05$). There was no statistical difference in blood loss between Groups A and B ($P>0.05$), but there were significant differences when Group A was compared with Groups C and E ($P<0.01$) (Table 3).

Morphological observation of knee joint

The knee joint Brittberg scores of animals were statistically different ($P<0.05$). The extent of joint destruction was the most serious in Group C, Group A was equivalent to Group D, and Group B was mild (Table 4, Figure 1).

Pathology

The articular cartilage of animals in each group were observed by HE staining (Figure 2). Cartilage of Group E and Group F were normal. Cartilage shallow surface, transitional layer, radiation layer, and calcified layer were clear. The tidal line was complete and distinct, and the structure of the bonding line was intact and undulating. The cartilage surface was smooth, without any defect and groove scar, and flat and round chondrocytes were arranged neatly (Figure 2E, 2F). The cartilage in Groups A, B, C, and D had various degrees of destruction. Four layers of cartilage in group A were fuzzy, the structure was disordered, and the tidal line was fuzzy and interrupted. The bonding line was not obvious and the surface of cartilage was uneven and defective. Some defects were deep in the radiation layer and calcified layer. The number of chondrocytes in each layer increased, the arrangement was disordered, and the staining decreased (Figure 2A). Compared with Group A, the pathological changes in Group B were smaller. The boundary of each layer was obscure and the tidal line was continuous. At the same time, the bonding line was not obvious and the cartilage surface was uneven. Some of the defects were deep and involved the radiation layer or calcified layer, and staining decreased (Figure 2B). Cartilage destruction in Group C was the most serious, and the layers could not be distinguished. The structure level was disordered and the tide line was fuzzy and interrupted. The adhesive line was not

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Table 4. Comparison of knee joint Brittberg scores of macaca fascicularis in each group (n=3).

Group	0	1	2	3	4	H	P
Group A	0	0	n=2	n=1	0	15.42	0.009
Group B	0	n=2	n=1	0	0		
Group C	0	0	0	n=3	0		
Group D	0	0	n=2	n=1	0		
Group E	n=3	0	0	0	0		
Group F	n=3	0	0	0	0		

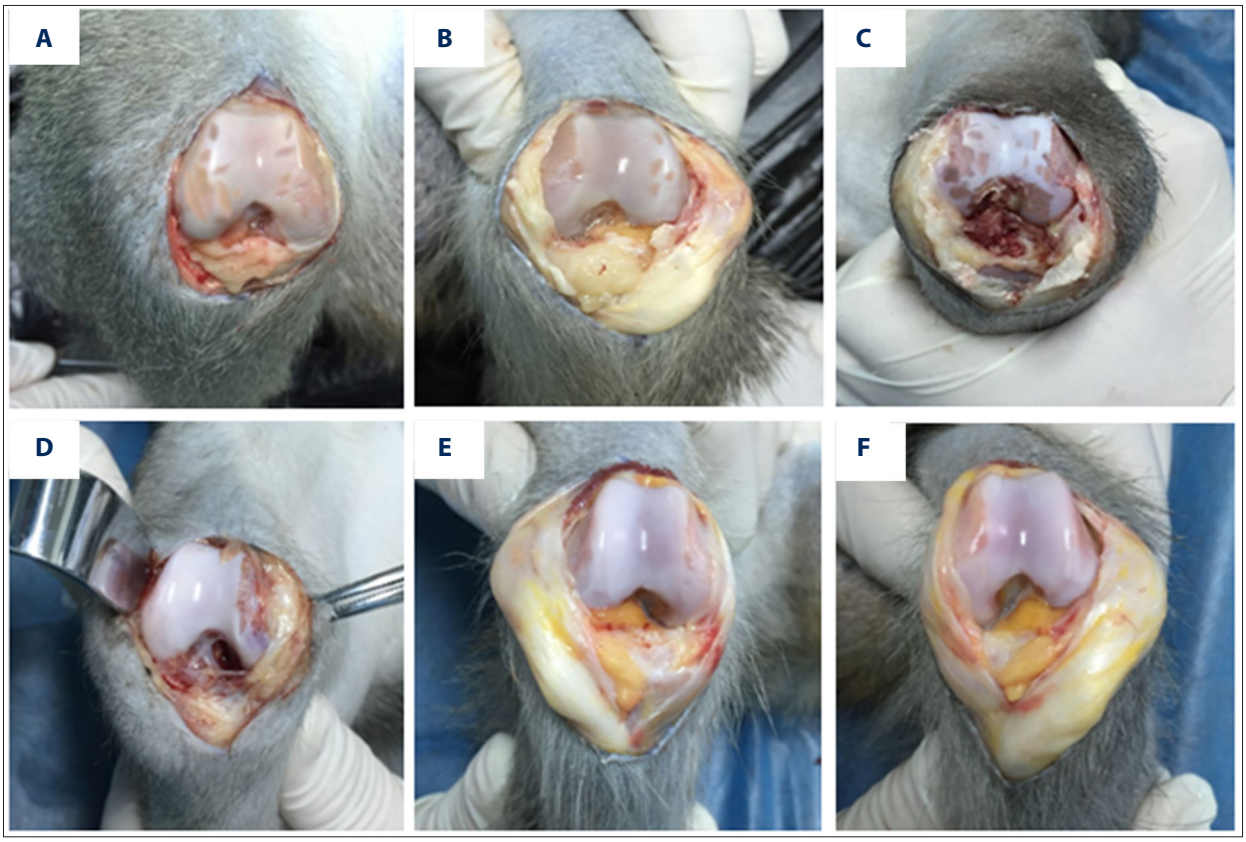

Figure 1. Knee joint morphology of *Macaca fascicularis*. (**A**) The cartilage of the medial and lateral condyle of knee joint were damaged to different degrees, and the gloss of articular cartilage was lower than normal in Group A; (**B**) The abrasion of articular cartilage mainly focused on the medial and lateral condyle in Group B; (**C**) The joint surface abrasion and destruction were serious, the anterior cruciate ligament, medial collateral ligament, and medial meniscus were missing, and the articular cartilage was dull in Group C; (**D**) The abrasion of articular cartilage mainly concentrated on unilateral femoral condyle, and the brightness of articular cartilage was lower than normal cartilage in Group D; (**E**) The articular cartilage was normal in Group E; (**F**) Normal articular cartilage of *Macaca fascicularis* in Group F.

obvious, the cartilage surface was defected, and some defects involved the radiation layer or calcified layer. The chondrocytes in each layer were disordered and the staining obviously decreased (Figure 2C). The lesions in Group D were similar to those in Group A (Figure 2D). By comparing the modified Mankin score of each group, we found that there were significant differences (P<0.01) among Group A, B, C, E, and F, and there was no significant difference between Group A and Group D (P>0.05) (Table 5).

Expression of WBC, IL-1β, and TGF-β1 in synovial fluid

The levels of WBC, IL-1β, and TGF-β1 in synovial fluid were detected at 8 weeks after the modeling operation. There were

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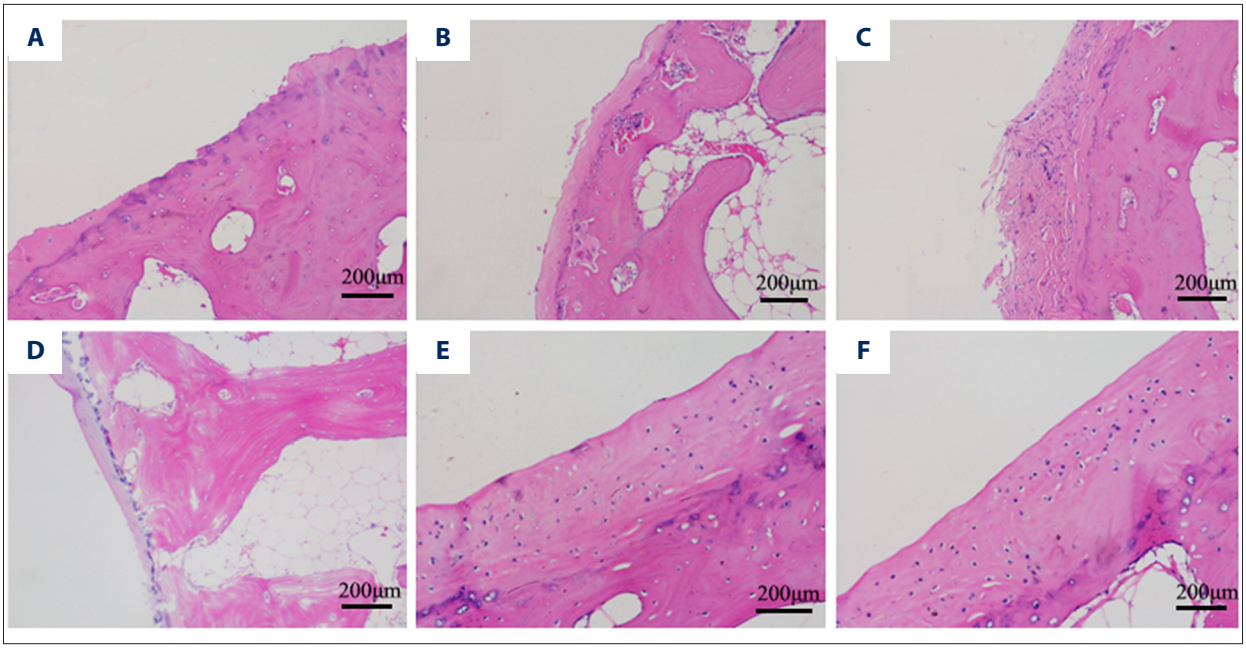

Figure 2. HE staining of articular cartilage in *Macaca fascicularis* of each group. (**A**) Articular cartilage surface were defected, cartilage destruction involved radiation layer, and tidal line was interrupted and fuzzy in Group A. (**B**) Articular cartilage surfaces were partly destroyed, did not involve cartilaginous lower layer, and the tidal lines were blurred in Group B. (**C**) Articular cartilage surface were defected and structure was disordered in Group C. (**D**) Articular cartilage surface were damaged to different degrees, cartilage destruction involved radiation layer, and the tidal line was interrupted and blurred in Group D. (**E**) The articular cartilage were intact and basically normal in Group E. (**F**) Articular cartilage surface were smooth and the structure levels were obvious.

Table 5. Comparison of modified Mankin score of articular cartilage in macaca fascicularis after 8 weeks ($\bar{\chi}$±s, n=5).

Group	Modified Mankin score	F	P
Group A	8.2±0.84	191.04	0.001
Group B	3.4±0.58a		
Group C	11.4±1.14[ab]		
Group D	8.8±0.84[c]		
Group E	0.4±0.55[abcd]		
Group F	0.2±0.48[abcd]		

[a] P<0.01 *vs.* group A; [b] P<0.01 *vs.* group B; [c] P<0.01 *vs.* group C; [d] P<0.01 *vs.* group D.

significant statistical differences among Groups A, B, C, and D (P<0.01) in WBC, but no statistical difference was found between Groups E and F (P>0.05). For IL-1β, there were significant statistical differences among Groups A, B, C, and D (P<0.01), but we found no significant difference between Groups E and F (P>0.05). There were significant statistical differences among Groups A, B, C, and D (P<0.01) in TGF-β1, but no significant difference was found between Groups E and F (P>0.05) (Figure 3).

Expression of COL-II, ACAN, and MMP-13 mRNA in articular cartilage

Expressions of COL-II, ACAN, and MMP-13 genes were detected by RT-qPCR technique. Except for between groups A and D and Groups D and F, there were significant differences in other groups (P<0.05) in COL-II. As for ACAN, the same as COL-II, there were significant differences in other groups (P<0.05) except for between Groups A and D and Groups D and F. When related to MMP-13, there were significant differences among all groups except for between Groups A and D and Groups D and F (P<0.05) (Figure 4).

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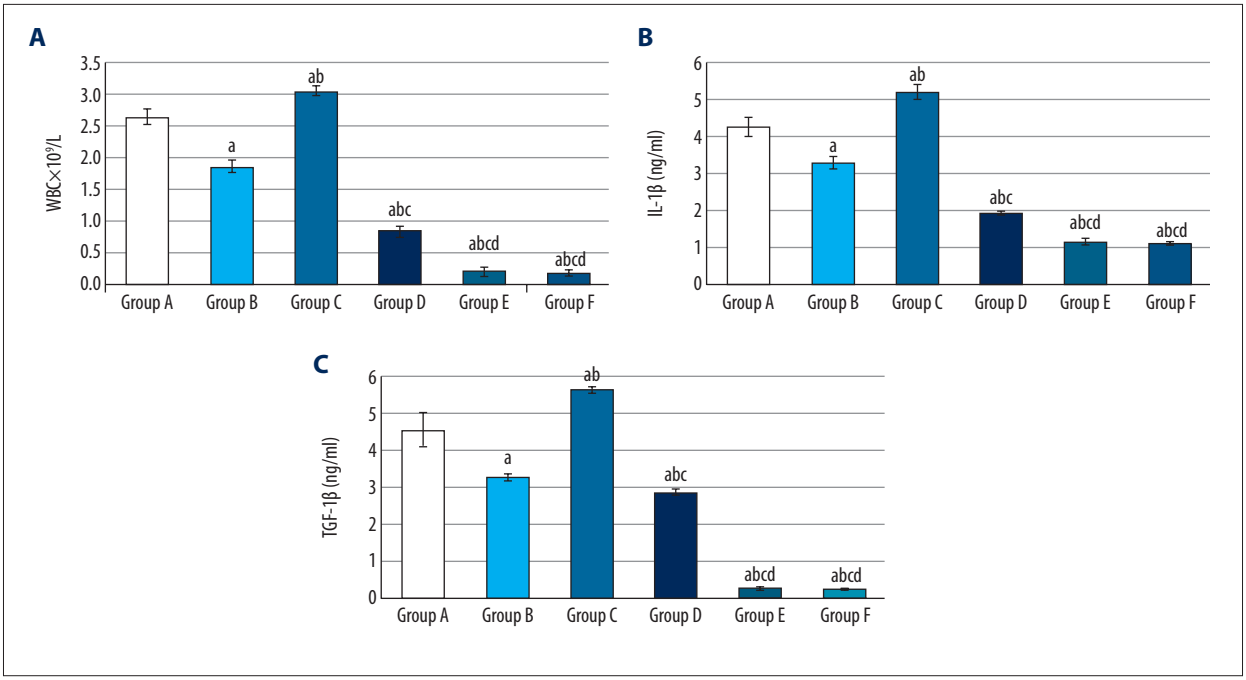

Figure 3. Expression of WBC, IL-1β, and TGF-β1 in synovial fluid. (**A**) Expression of WBC. (**B**) Expression of IL-1β. (**C**) Expression of TGF-β1. a P<0.01 *vs.* Group A; b P<0.01 *vs.* Group B; c P<0.01 *vs.* Group C; d P<0.01 *vs.* Group D.

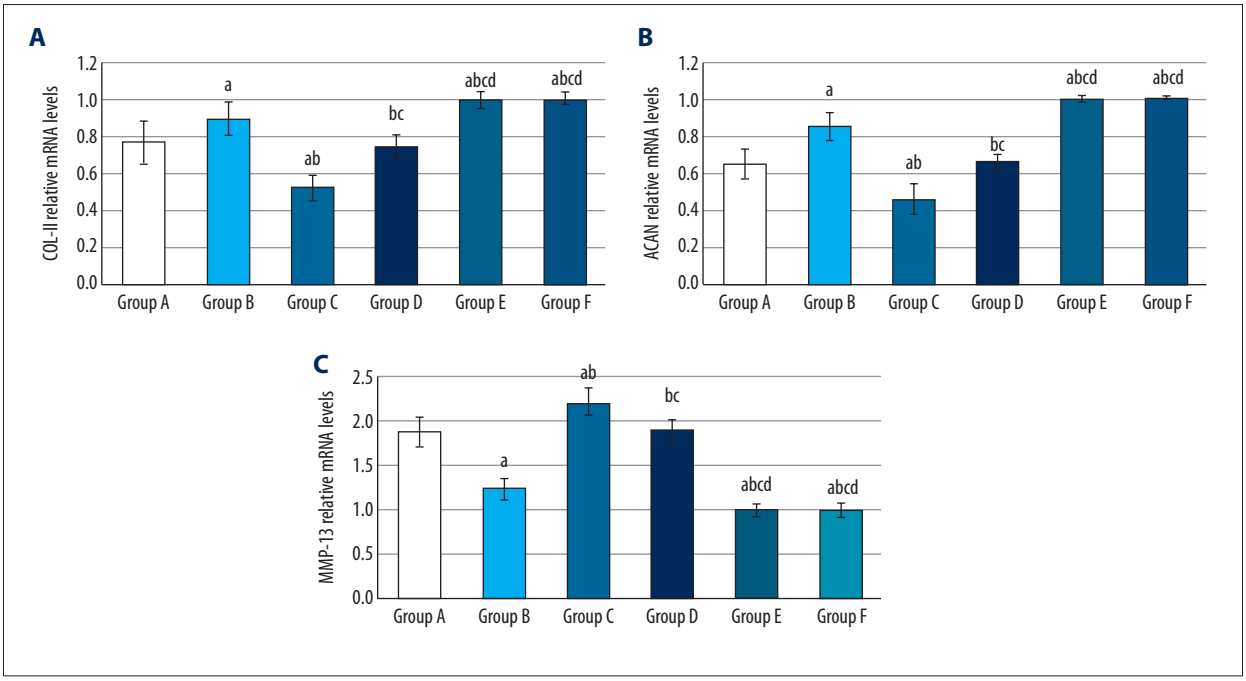

Figure 4. Expression of COL- II, ACAN and MMP-13 mRNA in articular cartilage. (**A**) Expression of COL-II. (**B**) Expression of ACAN. (**C**) Expression of MMP-13. a P<0.01 *vs.* Group A; b P<0.01 *vs.* Group B; c P<0.01 *vs.* Group C; d P<0.01 *vs.* Group D.

Expression of COL- II, ACAN, and MMP-13 in articular cartilage by Western blot

Expressions of COL-II, ACAN, and MMP-13 genes were detected by Western blot analysis. There were significant differences among all groups (P<0.05) in COL-II except for between Groups A and D and Groups D and F. For ACAN and COL-II, there were significant differences among all groups (P<0.05) except for between Groups A and D and Groups D and F. For MMP-13, there were significant differences among all groups except for

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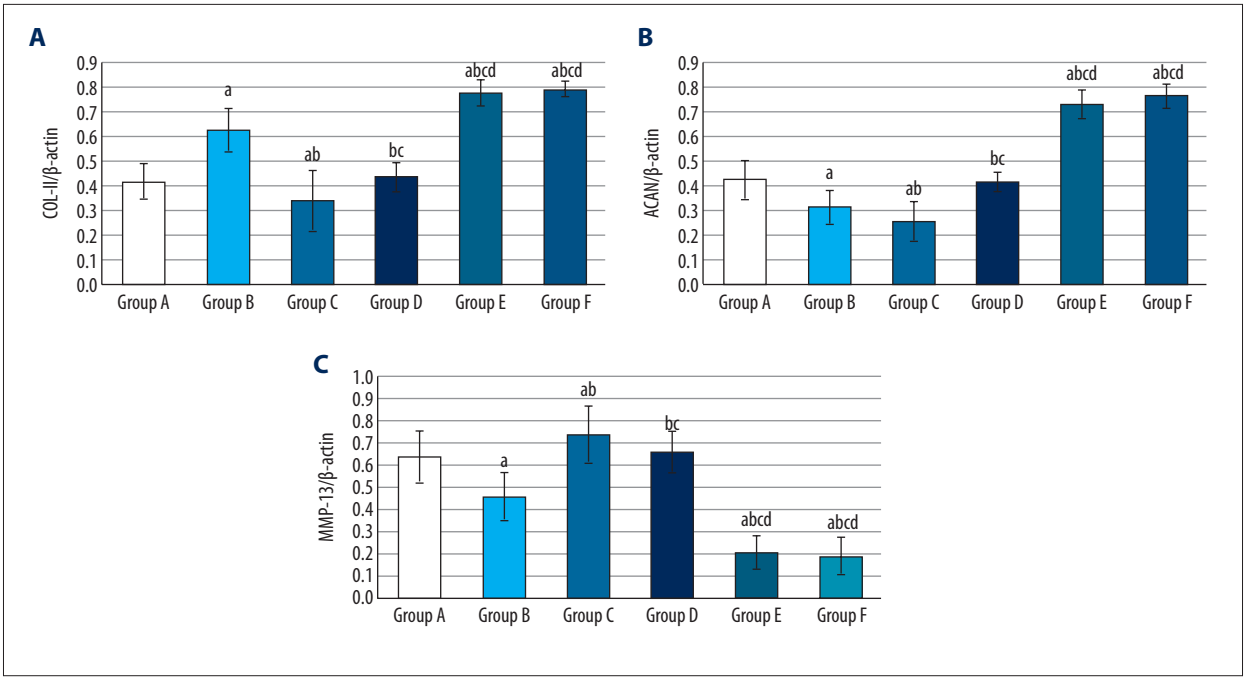

Figure 5. Expression of COL-II, ACAN, and MMP-13 protein in articular cartilage. (**A**) Expression of COL-II. (**B**) Expression of ACAN. (**C**) Expression of MMP-13. a P<0.01 *vs.* Group A; b P<0.01 *vs.* Group B; c P<0.01 *vs.* Group C; d P<0.01 *vs.* Group D.

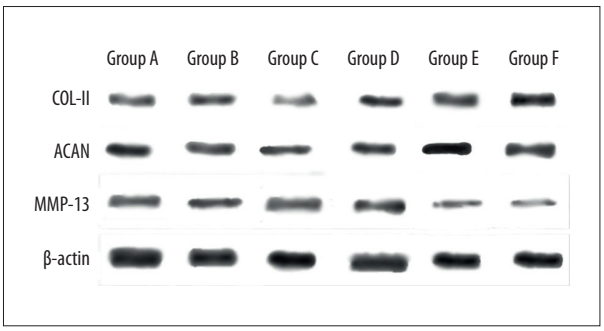

Figure 6. Western blot of COL- II, ACAN, MMP-13, and β-actin.

between Group A and D and Group D and F (P<0.05) (Figures 5, 6).

Discussion

According to the comparison of Brittberg and modified Mankin scores in Groups A, B, C, and D, the destruction of cartilage in group A was more serious than that in Group B and less serious than in Group C, and there were significant differences (P<0.05), but there was not a significant difference between Group A and Group D (P>0.05). Therefore, the cartilage destruction of the KOA model developed by modified Hulth combined with joint scratches at 8 weeks was closer to the spontaneous models. However, the articular cartilage in the KOA model developed by ACL transection alone showed abrasion and it may take longer to produce the same extent of joint damage found in a naturally degenerative model. The joint destruction caused by Hulth methods was obvious.

The expressions of WBC, IL-1β, and TNF-β1 in the synovial fluid of *Macaca fascicularis* were increased in Groups A, B, C, and D. There was no significant difference between Group A and Group D (P>0.05) or between Group E and Group F (P>0.05), but there were statistically significant differences among other groups (P<0.01). Therefore, the KOA models developed by the modified Hulth combined with the joint scratches at 8 weeks were similar to the spontaneous models in the changes of WBC and related cytokines in the synovial fluid. The biochemical and inflammatory changes in the synovial fluid were the most serious in models by Hulth, which were less serious in models developed by ACL transection.

The expression of COL- II and ACAN in the cartilage of *Macaca fascicularis* decreased, and MMP-13 increased in Groups A, B, C, and D. Except for Groups A, D, E, and F, the differences were statistically significant among the other groups (P<0.01). The expressions of COL-II, ACAN, and MMP-13 in the KOA model developed by the modified Hulth combined with the joint scratches at 8 weeks were similar to the spontaneous models.

Compared with other methods in morphology, in terms of biochemical and molecular biological levels, the models developed by modified Hulth combined with joint scratches modeling method are consistent and closely resemble the pathophysiological process of KOA.

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In KOA models, the spontaneous models are, to some extent, the closest to the naturally degenerative joint of the human body [16], but they often take more time to observe and test to determine whether they satisfied the model standard or not. Furthermore, the experimental results were also affected by many uncertainties, which reduced the reliability [17] and increased the consumption of experimental time and animals. Grynpas et al. [18] found that there was no significant difference in total collagen content between naturally degenerative rhesus monkeys KOA models and normal *Macaca fascicularis* based on hydroxyproline analysis. Transgenic animals, especially transgenic mice, have also rapidly developed in recent years and 135 subtypes [19,20] had been developed in the 2013 report. The single gene in the transgenic mouse model that had an effect on OA has been identified [12] and attracted researchers' attention. However, the pathogenesis of OA is caused by the combination of multiple genes and factors. The transgenic model cannot completely simulate the pathological process of human OA. The KOA model [21,22] can be rapidly created by injecting papain and collagenase into the joint cavity, which causes cartilage damage, and has been commonly used in cartilage pathology and drug treatment research [23]. However, because of the effects of anatomic site, joint size, and animal species, the drug dose required for modeling was also somewhat different [24,25].

Methods for modeling KOA by surgery have been widely used. Many studies have been published that surgically altered joint stability, including complete or partial meniscectomy, meniscus injury, ACL and PCL transection, medial or lateral collateral ligament transaction, osteotomy, implanting bone fragments in the joint cavity, and joint scratches. The mechanical balance of the joints was disturbed by one or more ways and led to KOA [11,12,17]. These methods were rapid, simple, highly repeatable, and have been widely used in KOA modeling. However, there is a lack of comparison among them due to the numerous methods used, and it is still difficult to select a quick and effective surgical method. The Hulth method requires cutting off the ACL, medial collateral ligament, and medial meniscus, and there was no damage of cartilage in the whole process [26]. This method completely undermined the stability of the joint compared with other modeling methods that can develop a KOA model in a shorter time, but the operation is more traumatic and there can be more bleeding during the operation. It also is more likely to cause joint dislocation, infection, and other complications after the operation. Therefore, it requires greater surgical skill. These models are suitable for research on joint replacement and cartilage repair according to the characteristics of Hulth models. Marijnissen et al. [27] performed surgical joint scratching on the weight-bearing area of the femoral condyle in beagle dogs, but it did not damage the subchondral bone. Compared with the models developed by ACL transaction, chondrocytes were lost and cartilage collagen was damaged after 10 weeks and chondrocytes accumulated around the femoral condyle. However, the production rate of MMP13 and other inflammatory factors were lower than in ACL transection models. Compared with previous methods of permanent damage such as ACL transection and others, this method of joint scratches reduces the effect of factors that promote long-term progression of KOA instability. It was more suitable for the observation of early effects, the therapeutic effect of KOA models, and repair and treatment of chondrocytes after injury.

The modeling method of Hulth surgery has been used for a long time [28] and the modeling effects have been recognized by many scholars. It has the advantages of fast modeling and obvious effect. But there are also some problems: it requires cutting off the ACL, medial collateral ligament, and medial meniscus, and the operation is more traumatic and requires higher surgical skill. It is extremely unstable in biomechanics and easily leads to dislocation of the knee joint after losing the stabilizing effect of the ACL and the medial collateral ligament. The joint loses its function of buffering after cutting off medial meniscus. The articular cartilage of experimental animals is easily abraded by postoperative activity, and infection is also a factor that can affect the outcome of the model. Joint dislocation can also lead to increased risk of infection due to the great trauma.

Based on the shortcomings of the Hulth modeling method, some scholars had also modified it as follows: (1) ACL transection [29–33] has been widely used and had less surgical trauma. The incidence of postoperative infection and joint dislocation was relatively low, the operation was relatively simple compared with Hulth, but it had a longer modeling time. This method was mainly used for cartilage pathology observation because it was less damaging [34]. (2) The medial meniscus was excised to prepare the KOA model and changed the biological line of the lower limb, increasing the varus degree of the knee, which led to varus stress and resulted in KOA lesions. Because this method causes imbalance of internal and external forces of the knee joint and there were some differences in behavior between experimental animals and humans, it takes a relatively long time to form early KOA lesions [35,36]. At the same time, it may have some differences in internal and external pathological changes of the knee joint. However, primates such as *Macaca fascicularis* are similar to humans in vertical movements, so further research on the effects of meniscus resection are needed to develop KOA models. (3) Some scholars combined the resection of ACL and medial meniscus, which reduced the modeling time to some extent. However, safety and effective KOA models [37] could not be rapidly developed. (4) In addition, there were other methods, such as transection of ACL combined with removal of medial collateral ligament, transection of medial collateral ligament and removal of medial meniscus, and the transection of medial collateral ligament alone [38]. Actually, all the above methods used different

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resectioning to arrange and combine the selected structures of the Hulth method, so as to study various modified Hulth methods. Among these methods, ACL transection was simple and minimally invasive, thereby causing relatively minimal damage to the physiological structure of animals and able to fully reflect the process of KOA lesion development, but it usually took more than 6 weeks to obtain the KOA model with early lesions [39]. Moskowitz et al. [40] performed partial removal of the meniscus of knee joints in rabbits to cause knee joint instability, and observed the pathological changes of OA at 6 months after the operation. This method can alleviate the joint injury, but it had a longer modeling time.

Compared with the Hulth method, the KOA models developed by joint scratches alone greatly reduced the damage to biomechanical stability of the knee joint. However, it was necessary to use weight-bearing intervention for the injured limb and it also took a long time to produce OA pathological changes. Marijnissen et al. [27] developed KOA models in beagle dogs by joint scratches, but the injured limbs were weight-bearing for 20 weeks after the operation. It was found that the biochemical characteristics of articular cartilage were very similar to clinical findings, and it was suitable for clinical observation. Meanwhile, a model suitable for early pathological changes in OA was obtained, but it also took a long time.

In the present study, the modified Hulth method combined with joint scratches method was used to develop the KOA models, which had a good effect, and the pathophysiological changes were close to the spontaneous KAO model of old *Macaca fascicularis*. On the one hand, the modified Hulth surgical intervention can shorten the time of modeling on the basis of joint scratches. The degree of articular cartilage lesions was similar to the natural degenerative model at 8 weeks after modeling, and the modeling time was relatively short. On the other hand, it reduced the risk of postoperative knee dislocation, infection, and damage to knee joint stability by reducing Hulth surgical trauma.

In the study of KOA treatment, Sungho Yun et al. [41] cut off the cruciate ligaments of the right hind leg of beagle dogs. Dogs were walked 10 min every day for 2 months, started 10 days after the operation. Then, they used bone marrow mesenchymal stem cells and platelet rich plasma to treat KAO. Although the modeling time was relatively long and they needed to increase the hours of the experimental staff, it also showed that the surgical models were suitable for the study of KOA treatment. Li Jiang et al. [42] used cynomolgus monkey mesenchymal stem cells for the treatment of *Macaca fascicularis* KOA models induced by collagenase, and showed a curative effect, but it took a long time to induce joint degeneration by collagenase. The study suggested that the *Macaca fascicularis* KOA model developed by modified Hulth method combined with joint scratches method is also suitable for the study of KOA treatment.

In this study, *Macaca fascicularis* were selected as model animals. The knee joint of *Macaca fascicularis* are more similar to human knee joints in structural organization compared than are commonly used laboratory animals such as mice and rabbits. *Macaca fascicularis* have behavioral patterns and biomechanical movements similar to those of humans, such as walking upright, jumping, and squatting, which makes the weight-bearing of the knee joint physiologically similar. Due to the origin of species, the gene sequence and social behavior of primates are similar to those of humans. Therefore, *Macaca fascicularis* were chosen for model animals in this study, which increased to value of our conclusions for fundamental research and clinical application when compared with other experimental animals.

The following deficiencies existed in this study: (1) It lacked large-scale modeling due to the objective conditions, such as experimental conditions, funds, time, and experimental animals. All the selected animals were males, and it is not clear if sex affects the modeling. Moreover, the pathological changes of the knee joints of various *Macaca fascicularis* models were not detected at multiple time points. (2) Due to the use of analgesics in the killing of animals before specimen collection, the interference of the analgesic to the results of the experiment was not eliminated. (3) Because of the limited experimental conditions, X-ray, MRI, and other imaging examinations were not carried out. (5) The observation time was relatively short, and the KOA model was developed by modified Hulth combined with the joint scratches, which still needs long-term observation and further study.

Conclusions

In conclusion, the KOA animal model has been an important way for researchers to study the pathogenesis and treatment of OA, which can be established quickly and effectively by the combination of modified Hulth surgery with joint scratches. This animal model conformed to the KOA pathophysiological changes, and the pathological changes of this model were the closest to the spontaneous models at 8 weeks. The operation method in this study had less trauma and reduced the destruction of knee joint biomechanical stability. It also reduced the incidence of operation complications and damage to animals under the successful establishment of animal model. Therefore, this animal model can meet the needs of KOA research.

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Conflicts of interests

None.

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References:

1. Giunta S, Castorina A, Marzagalli et al: Ameliorative effects of PACAP against artilage degeneration. Morphological, immunohistochemical and biochemical evidence from *in vivo* and *in vitro* models of rat osteoarthritis. Int J Mol Sci, 2015; 16(3): 5922–44
2. Szychlinska MA, Trovato FM, Di Rosa M et al: Co-expression and co-localization of cartilage glycoproteins CHI3L1 and lubricin in osteoarthritic cartilage: Morphological, immunohistochemical and gene expression profiles. Int J Mol Sci, 2016; 17(3): 359
3. Musumeci G, Aiello FC, Szychlinska MA et al: Osteoarthritis in the XXIst century: Risk factors and behaviours that influence disease onset and progression. Int J Mol Sci, 2015; 16(3): 6093–112
4. Xu Q, Chen B, Wang Y et al: The effectiveness of manual therapy for relieving pain, stiffness, and dysfunction in knee osteoarthritis: A systematic review and meta-analysis. Pain Physician, 2017; 20(4): 229–43
5. Lawrence RC, Felson DT, Helmick CG et al: Estimates of the prevalence of arthritis and other rheumatic conditions in the United States: Part II. Arthritis Rheum, 2008; 58(1): 26–35
6. Hunter DJ, Schofield D, Callander E: The individual and socioeconomic impact of osteoarthritis. Nat Rev Rheumatol, 2014; 10(7): 437–41
7. Hootman JM, Helmick CG: Projections of US prevalence of arthritis and associated activity limitations. Arthritis Rheum, 2006; 54(1): 226–29
8. Kotlarz H, Gunnarsson CL, Fang H, Rizzo JA: Insurer and out-of-pocket costs of osteoarthritis in the US: Evidence from national survey data. Arthritis Rheum, 2009; 60(12): 3546–53
9. Bitton R: The economic burden of osteoarthritis. Am J Manag Care, 2009; 15(8 Suppl.): S230–35
10. Goldring MB, Marcu KB: Cartilage homeostasis in health and rheumatic diseases. Arthritis Res Ther, 2009; 11(3): 224
11. McCoy AM: Animal models of osteoarthritis: Comparisons and key considerations. Vet Pathol, 2015; 52(5): 803–18
12. Lampropoulou-Adamidou K, Lelovas P, Karadimas EV et al: Useful animal models for the research of osteoarthritis. Eur J Orthop Surg Traumatol, 2014; 24(3): 263–71
13. Aldrian S, Zak L, Wondrasch B et al: Clinical and radiological long-term outcomes after matrix-induced autologous chondrocyte transplantation: A prospective follow-up at a minimum of 10 years. Am J Sports Med, 2014; 42(11): 2680–88
14. Mankin HJ, Dorfman H, Lippiello L: Biochemical and metabolic abnormalities in articular cartilage from osteoarthritic human hips III Distribution and metabolism of amino sugar-containing macromolecules. J Bone Jt Surg, 1981; 63(1): 131–39
15. Kraus VB, Huebner JL, Stabler T et al: Ascorbic acid increases the severity of spontaneous knee osteoarthritis in a guinea pig model. Arthritis Rheum, 2004; 50(6): 1822–31
16. Teeple E, Jay GD, Elsaid KA, Fleming BC: Animal models of osteoarthritis: Challenges of model selection and analysis. AAPS J, 2013; 15(2): 438–46
17. Stone AV, Vanderman KS, Willey JS et al: Osteoarthritic changes in vervet monkey knees correlate with meniscus degradation and increased matrix metalloproteinase and cytokine secretion. Osteoarthritis Cartilage, 2015; 7(10): 1780–89
18. Macrini TE, Coan HB, Levine SM et al: Reproductive status and sex show strong effects on knee OA in a baboon model. Osteoarthritis Cartilage, 2013; 21(6): 839–48
19. Grynpas MD, Gahunia HK, Yuan J et al: Analysis of collagens solubilized from cartilage of normal and spontaneously osteoarthritic rhesus monkeys. Osteoarthritis Cartilage, 1994; 2(4): 227–34
20. Little CB, Hunter DJ: Post-traumatic osteoarthritis: From mouse models to clinical trials. Nat Rev Rheumatol, 2013; 9(8): 485–97
21. Jiang L, Ma A, Song L et al: Cartilage regeneration by selected chondrogenic clonal mesenchymal stem cells in the collagenase-induced monkey osteoarthritis model. J Tissue Eng Regen Med, 2014; 8(11): 896–905
22. Udo M, Muneta T, Tsuji K et al: Monoiodoacetic acid induces arthritis and synovitis in rats in a dose- and time-dependent manner: Proposed model-specific scoring systems. Osteoarthritis Cartilage, 2016; 24(7): 1284–91
23. Artuzi FE, Langie R, Abreu MC et al: Rabbit model for osteoarthrosis of the temporomandibular joint as a basis for assessment of outcomes after intervention. Br J Oral Maxillofac Surg, 2016; 54(5): e33–37
24. Shuang F, Hou SX, Zhu JL et al: Establishment of a rat model of lumbar facet joint osteoarthritis using intraarticular injection of urinary plasminogen activator. Sci Rep, 2015; 20(5): 9828
25. Ogbonna AC, Clark AK, Malcangio M: Development of monosodium acetate-induced osteoarthritis and inflammatory pain in ageing mice. Age (Dordr), 2015; 37(3): 9792
26. Rogart JN, Barrach HJ, Chichester CO: Articular collagen degradation in the Hulth-Telhag model of osteoarthritis. Osteoarthritis Cartilage, 1999; 7(6): 539–47
27. Marijnissen AC, van Roermund PM, TeKoppele JM et al: The canine "groove" model, compared with the ACLT model of osteoarthritis. Osteoarthritis Cartilage, 2002; 10(2): 145–55
28. Hulth A, Lindberg L, Telhag H: Experimental osteoarthritis in rabbits. Acta Orthop Scand, 1970; 41(5): 522–30
29. Libicher M, Ivancic M, Hoffmann M, Wenz W: Early changes in experimental osteoarthritis using the Pond-Nuki dog model: Technical procedure and initial results of *in vivo* MR imaging. Eur Radiol, 2005; 15(2): 390–94
30. Murray MM, Fleming BC: Use of a bioactive scaffold to stimulate anterior cruciate ligament healing also minimizes posttraumatic osteoarthritis after surgery. Am J Sports Med, 2013; 41(8): 1762–70
31. Ouyang X, Wang J, Hong SD et al: Establishment of a rat model for osteoarthritis resulting from anterior cruciate ligament rupture and its significance. Exp Ther Med, 2015; 10(6): 2035–38
32. Pelletier JP, Boileau C, Brunet J et al: The inhibition of subchondral bone resorption in the early phase of experimental dog osteoarthritis by licofelone is associated with a reduction in the synthesis of MMP-13 and cathepsin K. Bone, 2004; 34(3): 527–38
33. Hamilton CB, Pest MA, Pitelka V et al: Weight-bearing asymmetry and vertical activity differences in a rat model of post-traumatic knee osteoarthritis. Osteoarthritis Cartilage, 2015; 23(7): 1178–85
34. Stok KS, Besler BA, Steiner TH et al: Three-dimensional quantitative morphometric analysis (QMA) for *in situ* joint and tissue assessment of osteoarthritis in a preclinical rabbit disease model. Plos One, 2016; 11(1): e0147564
35. Pashuck TD, Kuroki K, Cook CR et al: Hyaluronic acid versus saline intra-articular injections for amelioration of chronic knee osteoarthritis: A canine model. J Orthop Res, 2016; 34(10): 1772–79
36. Patel JM, Merriam AR, Culp BM et al: One-year outcomes of total meniscus reconstruction using a novel fiber-reinforced scaffold in an ovine model. Am J Sports Med, 2016; 44(4): 898–907
37. Joiner DM, Less KD, Van Wieren EM et al: Accelerated and increased joint damage in young mice with global inactivation of mitogen-inducible gene 6 after ligament and meniscus injury. Arthritis Res Ther, 2014; 16(2): R81
38. Ochiai N, Ohtori S, Sasho T et al: Brief report extracorporeal shock wave therapy improves motor dysfunction and pain originating from knee osteoarthritis in rats. Osteoarthritis Cartilage, 2007; 3(11): 1093–96
39. Pond MJ, Nuki G: Experimentally-induced osteoarthritis in the dog. Ann Rheum Dis, 1973; 32(4): 387–88
40. Moskowitz RW, Davis W, Sammarco J et al: Experimentally induced degenerative joint lesions following partial meniscectomy in the rabbit. Arthritis Rheum, 1973; 16(3): 397–405
41. Yun S, Ku SK, Kwon YS: Adipose-derived mesenchymal stem cells and platelet rich plasma synergistically ameliorate the surgical-induced osteoarthritis in Beagle dogs. J Orthop Surg Res, 2016; 11(1): 9
42. Jiang L, Ma A, Song L et al: Cartilage regeneration by selected chondrogenic clonal mesenchymal stem cells in the collagenase-induced monkey osteoarthritis model. J Tissue Eng Regen Med, 2014; 8(11): 896–905

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