

Original Article

Effects of low-intensity focused ultrasound on cartilage and synovium in an experimental model of osteoarthritis in rabbits

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Received May 10, 2016; Accepted July 26, 2016; Epub October 15, 2016; Published October 30, 2016

Abstract: Objective: To analyze the effects of low-intensity focused ultrasound (LIFUS) on cartilage and synovium and the underlying mechanism in the knee joints in an experimental rabbit model of osteoarthritis (OA). Methods: Adult male New Zealand white rabbits (n = 36) were divided into three groups (LIFUS, ACLT and Control). The LIFUS group and the ACLT group received unilateral anterior cruciate ligament transection (ACLT). Eight weeks after surgery, the LIFUS group was treated with LIFUS (300 mW/cm², 50% duty cycle, 1.5 MHz, 30 s per location, 6 locations per session, 5 sessions of 15 min daily), five times a week for 4 weeks. All rabbits were euthanized after the last treatment. The effects of LIFUS on gross morphology, cytokine and gene expression were evaluated by histology, ELISA and real-time quantitative PCR analyses, respectively. Results: Macroscopic morphologic assessment and histological observation showed that the damage to the cartilage and synovium in the LIFUS group was less than that in the ACLT group. Levels of IL-1 β , TNF- α and PGE₂ in the synovial fluids in the LIFUS group were lower than those in the ACLT group. Compared with the ACLT group, MMP-1, MMP-3, and MMP-13 mRNA in cartilage and synovium decreased significantly in the LIFUS group, while TIMP-1 mRNA increased significantly. Conclusion: LIFUS may protect against cartilage degradation and synovitis in rabbits with OA via a mechanism that may include regulation of MMP-1, MMP-3, MMP-13, TIMP-1, IL-1 β , TNF- α and PGE₂ gene expression in the cartilage and synovium.

Keywords: Osteoarthritis, cartilage, synovium, low-intensity focused ultrasound, rabbit

Introduction

Osteoarthritis (OA) represents a group of overlapping disorders in the whole joint caused by multiple confounding etiological factors. OA occurs primarily in older individuals and gradually leads to typical clinical symptoms such as pain, stiffness and limitation in functional activities [1]. In the USA, symptomatic knee OA occurs in approximately 10% of men and 13% of women aged 60 years or older [2], while in China, the prevalence is 55% in the elderly population aged over 60 (approximately 130 million people are currently affected by OA) [3]. OA is characterized by erosion and damage of the articular cartilage, formation of osteophytes at the margins and subchondral sclerosis, in addition to a battery of biochemical and morphologic alterations in the synovial membrane [4].

Several treatments are available for the management of OA, which involve the most conservative therapies for symptomatic relief in the early stages and surgical intervention in the final, severe stage [5]. Conservative methods include modifications of lifestyle, pharmacologic treatment and physical therapy, such as regular exercise, transcutaneous electrical neural stimulation, acupuncture and thermal modalities [6]. Conventional physical therapies could represent an alternative for bridging the gap between the disease onset and operative therapy. Some physical therapies are offered mainly to relieve or control symptoms but (influent) on damaged structure and function of cartilage and inflammatory process in advanced OA [7].

Ultrasound (US) is one type of physical therapy that has been employed for many decades

based on its documented safety and efficacy in numerous clinical applications and its capacity to induce a broad profile of systematic bio-effects [8]. Accumulating evidence from in vivo and in vitro studies as well as clinical research and systematic reviews have indicated the benefits of US in the treatment and management of OA [9]. Reports of animal trials and experimental studies consistently show that low-intensity pulsed unfocused therapeutic US in the range of 200-400 mW/cm² optimally promotes the cartilage and chondrocyte metabolism, stimulates the chondrocyte proliferation of suppresses cartilage degeneration [10].

Focused US, which is also one modality of therapeutic ultrasound, can be applied as high-intensity focused ultrasound (HIFUS) and low-intensity focused ultrasound (LIFUS). The former is an approved therapeutic procedure to treat tumors, such as uterine fibroids, breast cancer, and prostate cancer, while the latter utilizes low-energy US waves (intensity range, 1-1,000 mW/cm²; frequency range, 1-3 MHz) that pass through the skin and skull without surgery and which can be focused almost anywhere in the body, but especially in the brain [11]. Currently, low-power focused US is regarded as a safe alternative for brain neuromodulation [12]; however, studies of the application of LIFUS in other tissues or disorders are rare. LIFUS has positive effects on inflammation and tissue restoration [11]. Considering the known characteristics and mechanisms of LIFUS, we hypothesized that LIFUS may be effective for suppressing cartilage degeneration and controlling synovitis in OA.

In this study, we investigated the effect of LIFUS on cartilage and synovium in the knee joints of an experimental rabbit model of OA induced by transection of the anterior cruciate ligament (ACL) and studied the mechanisms.

Materials and methods

Animal model and treatment

All procedures were conducted according to the guidelines of the Institutional Animal Care and Use Committee of West China School of Medicine of Sichuan University (China), and were approved by the Animal Experimentation Ethics Committee of the West China School of Medicine of Sichuan University. Thirty-six adult

male New Zealand white rabbits (Science Center of West China hospital, Chengdu, China) aged 7 months and weighing approximately 3 to 3.5 kg were randomly divided into three groups (n = 12 per group): ACLT, LIFUS and Control. Animals were housed individually (cage dimensions, 70×70×50 cm) under a specific pathogen-free conditions (controlled temperature, 24±3°C and humidity, 45±15%) and fed a standard laboratory diet.

Surgical anterior cruciate ligament transection of the left knee was performed in the LIFUS and ACLT groups [13]. This model of surgically-induced OA produces cartilage lesions similar to those observed in human OA [14]. The 24 rabbits from the two groups were anesthetized by intravenous injection of sodium pentobarbital (30 mg/kg) via the marginal ear vein. The left knee was shaved and disinfected with iodine before incision of the medial patella through the skin and fascia. The patella was dislocated laterally, and the knee was placed in full flexion. After the articular capsule was opened, the anterior cruciate ligament (ACL) was transected with ophthalmic scissors. The joint was closed after irrigation with sterile saline. The capsule and the skin were closed by nylon mattress. The animals were returned to the cage after analepsia without immobilization. The Control group did not receive any surgery or treatment throughout the whole course of this experiment.

Eight weeks after surgery, 12 rabbits from the LIFUS group received low-intensity focused ultrasound treatment five times a week for 4 weeks. Before every treatment, the left knee of the rabbit was shaved. The specifications of the low-intensity focused ultrasound used (a focused ultrasound device, CZG200 made by Chongqing Haifu Medical Technology Co., Ltd, Chongqing, China) are based on previous studies and adjusted moderately according to requirements of this study [12]. All rabbits were euthanized after the last treatment.

Macroscopic morphologic assessment of cartilage

Macroscopic scoring of each left medial and lateral femoral condyle (MFC and LFC) and the medial and lateral tibial plateaus (MTP and LTP) was performed in a blinded manner according to previously described scoring systems [15].

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Digital images (Canon EOS1100D) of specimens with a scale marker were used to guide the selection of sections containing the most severe lesions in each compartment. The criteria used were as follows: 0 (normal), 1 (focal surface roughness), 2 (widespread surface irregularity), 3 (beginning surface fibrillation), 4 (severe surface fibrillation), 5 (beginning erosion), 6 (severe erosion); 7 (slight ulceration), and 8 (severe ulceration). India ink was not used in applying this scoring system, which is recommended by the Osteoarthritis Research Society International (OARSI) [6]. The final score corresponds to the score of the most severe lesions. After macroscopic scoring, six samples of cartilage and synovium from each group were evaluated histologically and a further six samples of each tissue were used to perform gene expression analysis.

Histological examinations of cartilage and synovium

Specimens of the condyle of the femur and tibial plateau from six rabbits per group were fixed in 10% neutral buffered formalin, and then decalcified in 10% ethylenediamine tetraacetic acid (EDTA) at 10°C for 5 weeks. After dehydration, all samples were embedded in paraffin, and cut into 5 µm thick sections for histological evaluation. Sections were stained with Safranin O-fast green and hematoxylin and eosin (HE) and then evaluated according to modified Mankin's grading system [16]. Specimens of synovium of the suprapatellar pouch that were distant from the arthrotomy site were fixed in 10% neutral buffered formalin, embedded in paraffin, and cut into 4 µm thick sections for histological evaluation. Sections were stained with HE and the synovial alterations were assessed according to the system of histopathological assessment of OA synoviopathy recommended by the OARSI [17]. The final score corresponds to the score of the most severe lesions. All sections were graded by two independent observers blinded to the treatment groups.

Gene expression analysis of cartilage and synovium

The cartilage tissues from the femoral condyle, tibial plateau and synovium were harvested from the left knees and total RNA was extracted using Trizol reagent. For first strand cDNA

synthesis, 1 µg of RNA was reverse transcribed using a Moloney murine leukemia virus reverse transcriptase cDNA synthesis kit (Promega, USA) at 37°C for 1 h. Gene expression of MMP-1, MMP-3, MMP-13, and TIMP-1 was quantitated by real-time quantitative PCR using the iCycler apparatus system (Bio-Rad, USA). The quantitative measurement of the expression of each gene was normalized to the amount of a housekeeping gene (β -actin). To confirm amplification specificity, the PCR products were subjected to melting curve analysis, and the data were analyzed and quantified using software for the calculation of relative expression in real-time PCR with a pairwise fixed reallocation randomization test (Relative Expression Software Tool). The Δ Ct value for each sample was obtained by subtracting the Ct value of β -actin.

Enzyme linked immunoassay (ELISA) of synovial fluids

Saline solution (0.5 ml) was injected into the joint space through the patellar tendon. After washing, the needle was reinserted and the synovial fluid was aspirated; samples were stored at -80°C until the levels of interleukin-1 β , tumor necrosis factor- α and prostaglandin E2 (PGE2) were assessed using ELISA kits (Boster Co., Wuhan, China).

Statistical analysis

The data obtained in this study were analyzed with SPSS 17.0 software for Windows. All data were expressed as the mean \pm SD and analyzed using Student's *t*-test, with *P* < 0.05 considered to indicate statistical significance.

Results

Effect of LIFUS on macroscopic morphologic assessment of cartilage

All specimens from the ACLT and LIFUS groups (*n* = 12 per group) showed complete transection of the ACL at the time of euthanization. In the ACLT and LIFUS groups, the most severe area (fibrillation, erosion and ulceration) of cartilage degeneration was observed predominantly in the MFC. Lesions in the femoral condyles were more severe than those in the tibial plateau. Generally, condyles and plateaus in the LIFUS group showed less severe cartilage

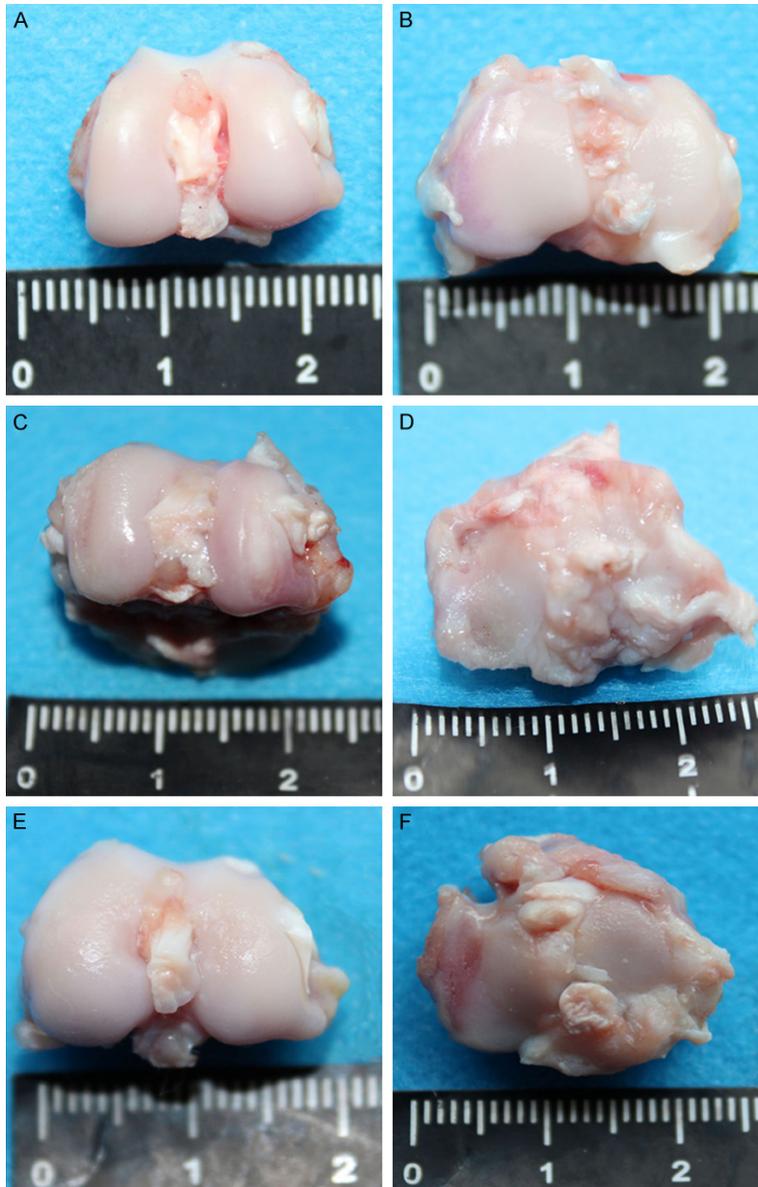


Figure 1. A: Femoral condyle of control group, intact surface of MFC and LFC; B: Tibial plateau of control group, intact surface of MTP and LTP; C: Femoral condyle of ACLT group, severe erosion extending into the deep layers on MFC with complete cartilage ulceration with subchondral bone exposure on LFC; D: Tibial plateau of ACLT group, slight ulceration on the caudal and anterior aspect of the MTP with severe fibrillation and adhesions on LTP; E: Femoral condyle of LIFUS group, severe surface fibrillation occurred on the MFC and LFC; F: Tibial plateau of LIFUS group, erosion starting to appear on the MTP with slight fibrillation on the LTP.

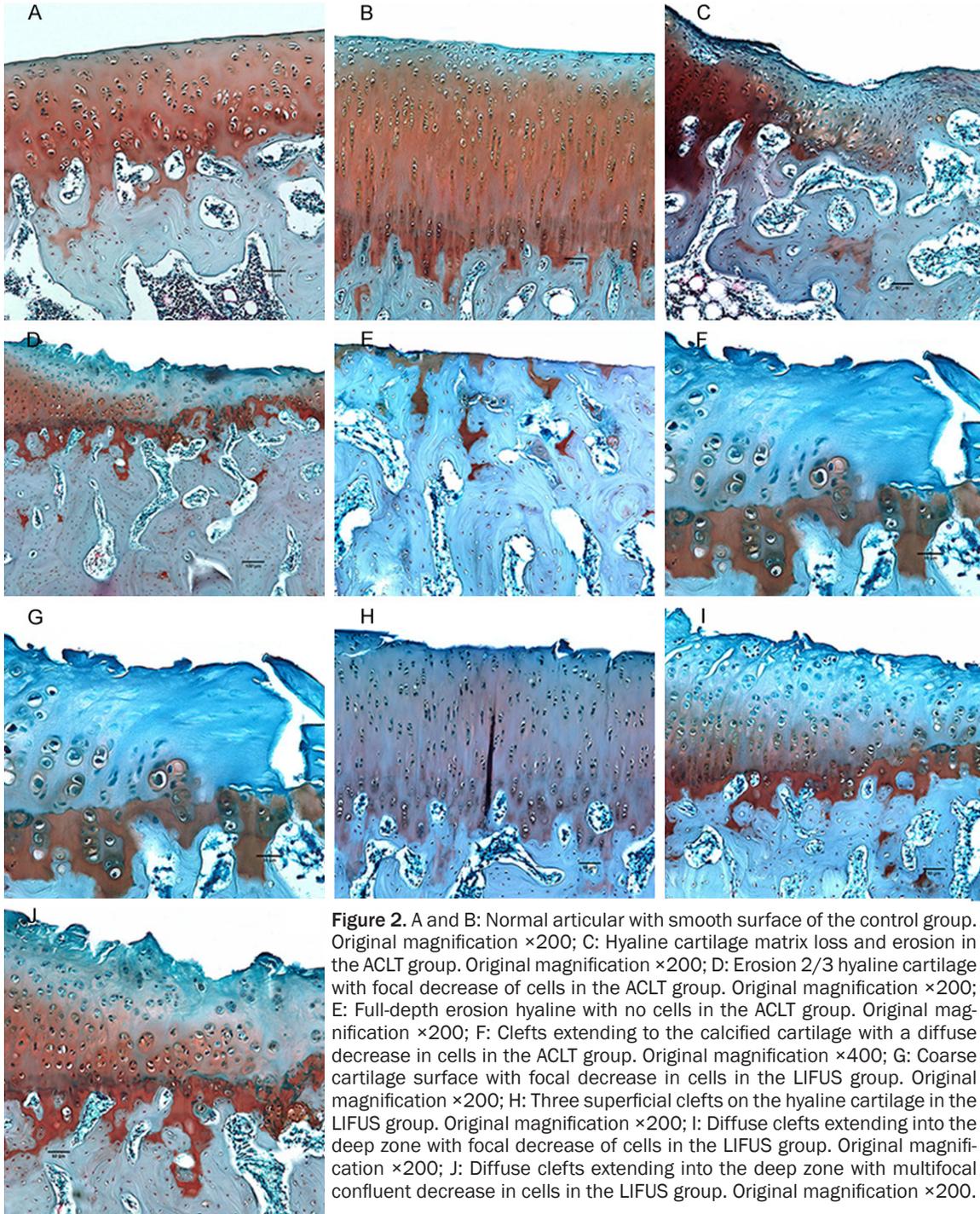
damage than those in the ACLT group. Severe ulceration (score 8) and slight ulceration (score 7) of the MFC were observed in three ACLT rabbits and one LIFUS rabbit, respectively. Severe erosion (score 6) of the MFC was observed in four ACLT rabbits and two LIFUS rabbits. Only one rabbit in the ACLT group showed severe fibrillation (score 4) on the MFC surface, while

the MFC of 50% (6/12) the rabbits in the LIFUS group scored 4. This difference was statistically significant ($P < 0.01$). The lesions in the MTP of the LIFUS group exhibited significantly lower grades of than those in the ACLT group ($P < 0.05$). The extent and grade of the damage to the cartilage in other compartments (LFC and LTP) in the LIFUS group were less severe than in the ACLT group, although this difference was not statistically significant ($P < 0.05$). **Figure 1** shows the macroscopic characteristics of the three groups.

Histological examinations of cartilage and synovium

Specimens of cartilage from the ACLT group ($n = 6$) exhibited morphologic changes, including loss of staining in all the hyaline cartilage of the condyle and plateau, full-depth erosion of the hyaline cartilage as well as calcification and hypocellularity of the cartilage to the subchondral bone. Specimens of cartilage from the LIFUS group ($n = 6$) showed loss of hyaline cartilage from the upper two-thirds of the superficial zone, fibrillation, clusters of chondrocytes, and reduced matrix staining in the radial layer. Lesions in the femoral condyles were more severe than those in the tibial plateau, which was consistent with the results of macroscopic morphology examinations. A significant decrease in the severity of lesions was observed in the MFC and MTP of the LIFUS group ($P < 0.05$). The decrease in the severity of structural changes and loss of matrix shown in Safranin O staining were the main reason for the reduction in the histological score. **Figure 2** shows the histological characteristics of the cartilage in the three groups.

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Specimens of synovium from the ACLT group (n = 6) exhibited progression from moderate to severe lesions, including synoviocyte proliferation and hypertrophy, fibroblast proliferation, lymphoplasmacytic infiltration, proliferation of blood vessels, fragments of bone matrix partially embedded in the synovium and marked

hemosiderosis with many macrophages containing hemosiderin. Specimens of cartilage from the LIFUS group (n = 6) showed progression from slight to moderate lesions. There were significant differences between the ACLT group and the LIFUS group in half of the inflammation parameters studied. The decrease in

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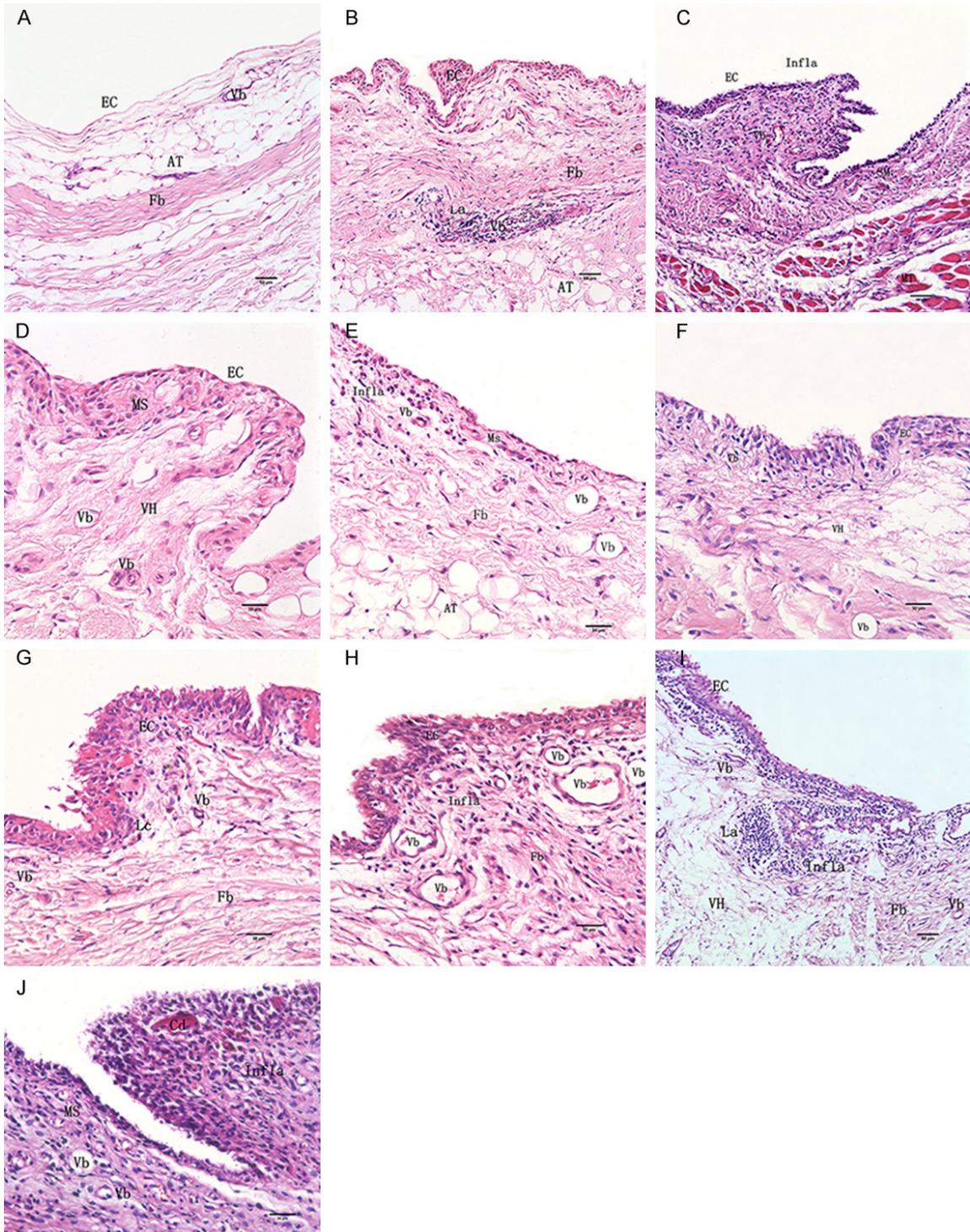


Figure 3. A: Normal synovial membrane in the control group; B: Synovitis in the LIFUS group, moderate synoviocyte proliferation and slight hyperplasia, with moderate lymphoplasmacytic infiltration around vessels, slight villous hyperplasia and slight fibroblast proliferation, and no cells containing hemosiderin pigments; C: Synovitis in the ACLT group, moderate synoviocyte proliferation and moderate hyperplasia, with moderate diffuse lymphoplasmacytic infiltration slight villous hyperplasia and moderate fibroblast proliferation, moderate proliferation of blood vessels and many cells containing hemosiderin pigments; D: Synovitis in the LIFUS group, moderate proliferation of synoviocytes with no hyperplasia, slight lymphoplasmacytic infiltration, some small villi and moderate proliferation of blood vessels; E: Synovitis in the LIFUS group, slight proliferation of synoviocytes with no hyperplasia, slight lymphoplasmacytic infiltration, moderate fibroblast proliferation and slight proliferation of blood vessels; F: Synovitis in the LIFUS

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group, moderate synoviocyte proliferation and hyperplasia, slight lymphoplasmacytic infiltration, moderate multifocal villous hyperplasia; G: Synovitis in the ACLT group, severe hyperplasia and hypertrophy of synoviocytes, with severe fibroblast proliferation and many cells containing hemosiderin pigments; H: Synovitis the ACLT group, severe synoviocyte proliferation and hyperplasia, severe fibroblast proliferation, diffuse lymphoplasmacytic infiltration, and severe proliferation of blood vessels; I: Synovitis in the ACLT group, severe synoviocyte proliferation and hyperplasia, moderate fibroblast proliferation, slight proliferation of blood vessels and lymphoplasmacytic aggregation; J: Synovitis in the ACLT group, partial embedding of a piece of bone matrix in the synovium; A-C. Original magnification $\times 200$; D-J. Original magnification $\times 400$. AT: Adipose tissue; Cd: Cartilage/bone-detritus; EC: Epithelioid cells; Fb: Fibroblast; Infla: Inflammation; Lc: Lymphocyte; Ms: Synovial membrane; Vb: Blood vessel; VH: Villous hyperplasia.

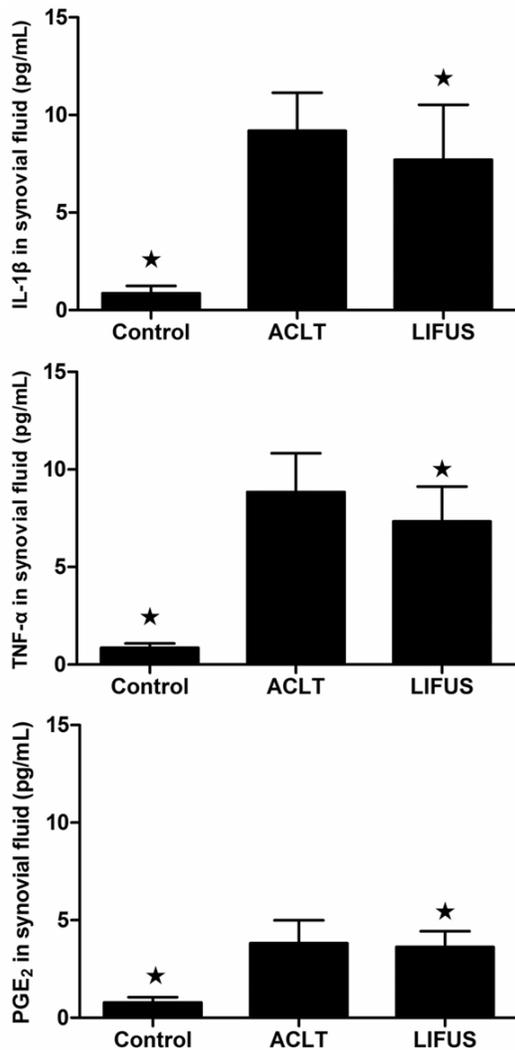


Figure 4. Levels of IL-1 β , TNF- α and PGE₂ in synovial fluid of the three groups. * $P < 0.05$, ** $P < 0.01$.

the severity of synoviocyte proliferation and hypertrophy, villous hyperplasia and proliferation of blood vessels in the synovial stroma and hemosiderosis are the main reason for the reduction of the histological score. **Figure 3** shows the histological characteristics of the synovium in the three groups.

ELISA analysis of inflammatory molecules

Figure 4 show the levels of IL-1 β , TNF- α and PGE₂ in synovial fluid from each group. Compared with the ACLT group, the levels of IL-1 β , TNF- α and PGE₂ were significantly decreased in the LIFUS group ($P < 0.05$), although the levels were significantly higher than those in the Control group ($P < 0.01$).

Gene expression in the cartilage and synovium

Figure 5 show the expression of MMP-1, MMP-3, MMP-13 and TIMP-1 in the cartilage and synovium. Compared with the ACLT group ($n = 6$), MMP-1, MMP-3, MMP-13 expression in cartilage and synovium was significantly reduced in the LIFUS group ($n = 6$) ($P < 0.05$), TIMP-1 ($P < 0.01$) expression was significantly increased in the LIFUS group. It is important to note that there was a significantly higher expression of MMP-1, MMP-3, MMP-13 and lower expression of TIMP-1 in the cartilage and synovium in the LIFUS group compared with that in the Control group. These results indicate that LIFUS directly suppressed the factors responsible for degeneration of the cartilage and synovium observed in the ACLT group, thereby preventing or reducing the cartilage damage caused by ACLT-induced OA.

Discussion

The purpose of our study was to investigate the effects of LIFUS on the cartilage and synovium of knee joints of rabbits with OA and to clarify the underlying mechanisms using a model of OA induced in rabbits by ACLT. This OA model, which has biochemical and pathological features similar to those observed in human OA, is one of the most commonly used to study the pathological changes and treatment options for OA [14]. This OA model. We implemented LIFUS 8 weeks after ACLT surgery, which is the time at which fibrosis, fissures or full-depth erosion of

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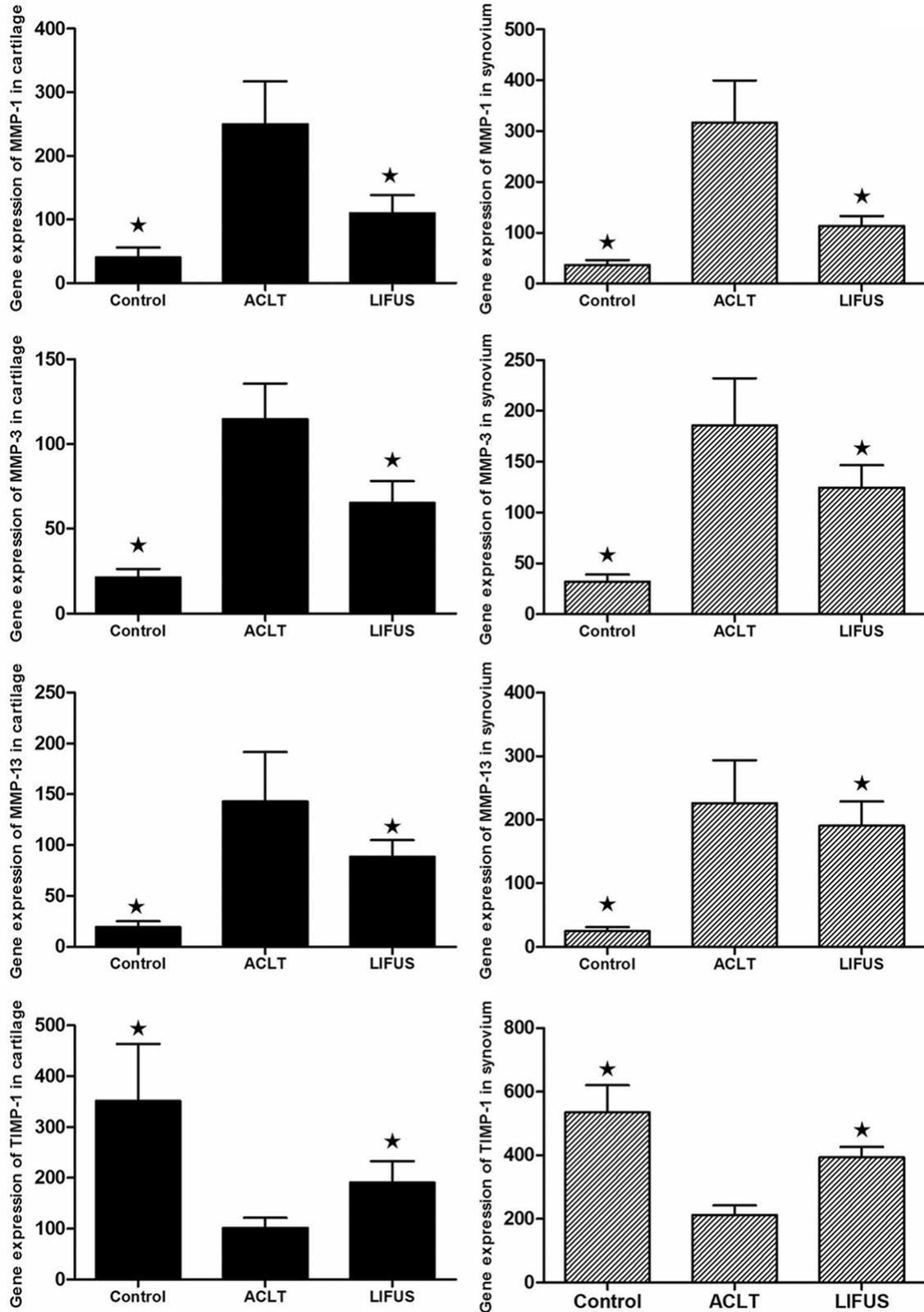


Figure 5. Expression of MMP-1, MMP-3, MMP-13 and TIMP-1 in cartilage and synovium of the three groups. * $P < 0.05$, ** $P < 0.01$.

the cartilage is observed in this model [18]. The cartilage lesions observed in each compartment are focal and usually centered in the medial femoral condyles and on the caudal aspect of the medial tibial plateau [19], which is consistent with the results of the macroscopic assessment conducted in our study.

In our study, Safranin O-fast green and HE staining revealed loss of proteoglycans in the femoral condyle and tibial plateau in this model. The modified Mankin's grading system was used to assess the effect of (LIFUS) on cartilage was assessed using Mankin's grading system, which offers proven reliability and consistency for grading histological changes observed in tissue sections. The histologically stained sections were scored by two independent, board-certified veterinary researchers who were blinded to the treatment groups. Compared with the ACLT group, there was significantly less damage in the MFC and MTP compartments in the LIFUS group, which suggests that LIFUS protects against cartilage degradation. However, we also found that LIFUS did not protect against erosion of the LFC and LTP cartilage. Interestingly, the extent and severity of lesions in articular cartilage observed by histologic examination were consistent with the macroscopic findings.

Inflammatory changes induced by ACLT consist of synovial effusion, hyperplasia of the synovial membrane and infiltration by inflammatory cells with sub-synovial fibrosis; these changes reflect the degenerative and inflammatory processes in joints [20]. HE staining is commonly used to analyze for the severity of synovitis. Various histological criteria and scores are used for the evaluation of the synovial membrane in this model [21]. In this study, we used the system of histological assessment of synovitis recommended by the OARSI, which captures the histological features of synoviopathy in OA [17]. Under normal physiological conditions the synovial lining consists of a thin layer of cells with phenotypic features of macrophages and fibroblasts. These cells and the underlying vascularized connective tissue stroma form a complex structure that is an important source of synovial fluid (SF) components that are essential for normal cartilage and joint function. Inflammation of the synovial membrane leads to synthesis and release of a wide variety of cytokines and chemokines, which

have catabolic effects on chondrocytes and play a role in the development of OA by promoting degradation of the articular cartilage [22]. In this study, we observed a moderate anti-inflammatory effect of LIFUS with a reduction in the severity of synovitis.

Proinflammatory cytokines, such as IL-1 β , TNF- α and PGE2 have a significant effect on chondrocytes, aggravating cartilage destruction [22], and are considered to be the principal inflammatory cytokines in OA. Furthermore, their expression in cartilage and SF may reflect the degree of inflammation in experimental OA. In the present study, analysis of SF showed that the expression of IL-1 β , TNF- α and PGE2 were increased in the ACLT group, while decreased expression was observed in the LIFUS group, corresponding to alleviated inflammation. This result indicates that LIFUS protects against cartilage degradation by interfering with the release of IL-1 β , TNF- α and PGE2 in the synovium or cartilage.

It is widely accepted that an imbalance in the synthesis and degradation of the articular matrix is a critical factor in the pathogenesis of OA [23]. Activation of chondrocytes is regarded to be an attempt to repair the cartilage matrix in early OA, which leads to upregulation of both anabolic and catabolic processes. However, this is not always effective and yields an inferior quality matrix that is more susceptible to degradation [24]. In the later stages of OA, synthesis of the matrix is decreased per chondrocyte, with a reduction in cell number that lead to the overall degradation of the cartilage [25]. In addition to the two catabolic factors (IL-1 β , TNF- α) previously mentioned, an array of proteases upregulated by IL-1 β and TNF- α are involved in the anabolic and catabolic processes of OA, particularly matrix metalloproteinases (MMPs) [26]. MMP-1 (interstitial collagenase), MMP-3 (stromelysin 1) and MMP-13 (collagenase-3) are capable of cleaving aggrecan core protein [27]. Tissue inhibitor of metalloproteinases (TIMP) is a key enzyme inhibitors, which specifically inhibits activated MMPs [28]. In our study, expression of MMP-1, MMP-3, and MMP-13 mRNA in cartilage and synovium decreased significantly in the LIFUS group compared with that in the ACLT group, while the expression of TIMP-1 mRNA increased significantly. Therefore, based on the results of our study, we cautiously suggest that LIFUS protects against cartilage

damage and the development of OA by inhibiting MMPs and the hydrolysis of TIMP-1.

Two previous studies using an animal model of OA have demonstrated increased hyaluronan absorption into rabbit knee joints under the influence of ultrasound phonophoresis [29]. However, investigations into the effects of LIFUS in OA and the underlying mechanism have not yet been reported. For reasons of safety, periods of LIFUS shorter than 30 s were used in this study to minimize the risk of thermal damage [30]. The safety of the LIFUS device relative to the concomitant use of MRI, especially when performed at 3 T/128 MHz, is crucial for applications in human subjects [30]. Unfortunately, we did not compare the effect of LIFUS with that of low-intensity pulsed unfocused therapeutic ultrasound at the same intensity and frequency and use MRI to guide the application of LIFUS in this study. Consequently, we are unable to determine which type of intervention provided better effects for OA. Therefore, further work should focus on providing a more detailed understanding of the effect of LIFUS in OA and the mechanism, as well as the application of MRI-guided LIFUS on OA, the safety of LIFUS in experimental animals and clinical OA patients and comparison of LIFUS with low-intensity pulsed unfocused therapeutic ultrasound applied at same intensity and frequency. Additionally, clinical trials of LIFUS for OA should be conducted in future.

The results of our limited study indicate that low-intensity focused ultrasound (LIFUS, 300 mW/cm², 50% duty cycle, 1.5 MHz, 30 s per location, 6 locations per session, 5 sessions of 15 min daily, 5 times a week for 4 weeks) provides protection against cartilage degradation and inflammation of the synovium in a rabbit model of OA. We cautiously suggest that this role may be achieved through the regulation of MMP-1, MMP-3, MMP-13, TIMP-1, IL-1 β , TNF- α and PGE2 gene expression in the cartilage and synovium. These findings provide a support for the use of LIFUS as a potential therapy in the management of knee joint OA.

Acknowledgements

The authors thank Key Laboratory of Rehabilitation Medicine in Sichuan (China) and the University-Town Hospital of Chongqing Medical University (China) for providing the

experimental facilities. They also acknowledge Chuan Liu, Xiaotian Yang, Yujing Zhou, Xiaofei We, Qiaodan Ji for providing assistance with these experiments.

Disclosure of conflict of interest

None.

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References

- [1] Felson DT. Osteoarthritis of the knee. *N Engl J Med* 2006; 354: 841-848.
- [2] Zhang Y and Jordan JM. Epidemiology of osteoarthritis. *Clin Geriatr Med* 2010; 26: 355-369.
- [3] Zhang J, Song L, Liu G, Zhang A, Dong H, Liu Z, Li X and Luo J. Risk factors for and prevalence of knee osteoarthritis in the rural areas of Shanxi Province, North China: a COPCORD study. *Rheumatol Int* 2013; 33: 2783-2788.
- [4] Abramson SB and Attur M. Developments in the scientific understanding of osteoarthritis. *Arthritis Res Ther* 2009; 11: 227.
- [5] Hochberg MC, Altman RD, April KT, Benkhalti M, Guyatt G, McGowan J, Towheed T, Welch V, Wells G and Tugwell P. American College of Rheumatology 2012 recommendations for the use of nonpharmacologic and pharmacologic therapies in osteoarthritis of the hand, hip, and knee. *Arthritis Care Res* 2012; 64: 465-474.
- [6] Conaghan PG, Dickson J and Grant RL. Guidelines: Care and management of osteoarthritis in adults: Summary of NICE guidance. *BMJ* 2008; 336: 502.
- [7] Guo H, Luo Q, Zhang J, Lin H, Xia L and He C. Comparing different physical factors on serum TNF- α levels, chondrocyte apoptosis, caspase-3 and caspase-8 expression in osteoarthritis of the knee in rabbits. *Joint Bone Spine* 2011; 78: 604-610.
- [8] Baker KG, Robertson VJ and Duck FA. A review of therapeutic ultrasound: biophysical effects. *Phys Ther* 2001; 81: 1351-1358.
- [9] Namazi H. Effect of low-intensity pulsed ultrasound on the cartilage repair in people with mild to moderate knee osteoarthritis: a novel molecular mechanism. *Arch Phys Med Rehabil* 2012; 93: 1882.
- [10] Li X, Li J, Cheng K, Lin Q, Wang D, Zhang H, An H, Gao M and Chen A. Effect of low-intensity

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- pulsed ultrasound on MMP-13 and MAPKs signaling pathway in rabbit knee osteoarthritis. *Cell Biochem Biophys* 2011; 61: 427-434.
- [11] Kortekaas MC, Kwok WY, Reijnen M, Huizinga TW and Kloppenburg M. In erosive hand osteoarthritis more inflammatory signs on ultrasound are found than in the rest of hand osteoarthritis. *Ann Rheum Dis* 2013; 72: 930-934.
- [12] Tyler WJ, Tufail Y, Finsterwald M, Tauchmann ML, Olson EJ and Majestic C. Remote excitation of neuronal circuits using low-intensity, low-frequency ultrasound. *PLoS One* 2008; 3: e3511.
- [13] Vignon E, Arlot M, Hartmann D, Moyen B and Ville G. Hypertrophic repair of articular cartilage in experimental osteoarthritis. *Ann Rheum Dis* 1983; 42: 82-88.
- [14] Pritzker KP. Osteoarthritis: Joint instability and OA: do animal models provide insights? *Nat Rev Rheumatol* 2011; 7: 444-445.
- [15] Wachsmuth L, Keiffer R, Juretschke HP, Raiss RX, Kimmig N and Lindhorst E. In vivo contrast-enhanced micro MR-imaging of experimental osteoarthritis in the rabbit knee joint at 7.1 T. *Osteoarthritis Cartilage* 2003; 11: 891-902.
- [16] Kikuchi T, Yamada H and Shimmei M. Effect of high molecular weight hyaluronan on cartilage degeneration in a rabbit model of osteoarthritis. *Osteoarthritis Cartilage* 1996; 4: 99-110.
- [17] Laverty S, Girard C, Williams J, Hunziker E and Pritzker K. The OARSI histopathology initiative-recommendations for histological assessments of osteoarthritis in the rabbit. *Osteoarthritis Cartilage* 2010; 18: S53-S65.
- [18] Chang DG, Iverson EP, Schinagl RM, Sonoda M, Amiel D, Coutts RD and Sah RL. Quantitation and localization of cartilage degeneration following the induction of osteoarthritis in the rabbit knee. *Osteoarthritis Cartilage* 1997; 5: 357-372.
- [19] Yoshioka M, Coutts RD, Amiel D and Hacker SA. Characterization of a model of osteoarthritis in the rabbit knee. *Osteoarthritis Cartilage* 1996; 4: 87-98.
- [20] Mehraban F, Tindal MH, Proffitt MM and Moskowitz RW. Temporal pattern of cysteine endopeptidase (cathepsin B) expression in cartilage and synovium from rabbit knees with experimental osteoarthritis: gene expression in chondrocytes in response to interleukin-1 and matrix depletion. *Ann Rheum Dis* 1997; 56: 108-115.
- [21] Elmali N, Ayan I, Türköz Y, Mizrak B, Germen B and Bora A. Effect of caffeic acid phenethyl ester on cartilage in experimental osteoarthritis. *Rheumatol Int* 2002; 22: 222-226.
- [22] Hui AY, McCarty WJ, Masuda K, Firestein GS and Sah RL. A systems biology approach to synovial joint lubrication in health, injury, and disease. *Wiley Interdiscip Rev Syst Biol Med* 2012; 4: 15-37.
- [23] Dean DD, Azzo W, Martel-Pelletier J, Pelletier JP, Woessner JF Jr. Levels of metalloproteases and tissue inhibitor of metalloproteases in human osteoarthritic cartilage. *J Rheumatol* 1987; 14: 43-44.
- [24] Umlauf D, Frank S, Pap T and Bertrand J. Cartilage biology, pathology, and repair. *Cell Mol Life Sci* 2010; 67: 4197-4211.
- [25] Mankin HJ, Dorfman H, Lippiello L and Zarins A. Biochemical and metabolic abnormalities in articular cartilage from osteoarthritic human hips. *J Bone Joint Surg Am* 1971; 53: 523-537.
- [26] Goldring M, Otero M, Tsuchimochi K, Ijiri K and Li Y. Defining the roles of inflammatory and anabolic cytokines in cartilage metabolism. *Ann Rheum Dis* 2008; 67: iii75-iii82.
- [27] Little CB, Hughes CE, Curtis CL, Janusz MJ, Bohne R, Wang-Weigand S, Taiwo YO, Mitchell PG, Otterness IG and Flannery CR. Matrix metalloproteinases are involved in C-terminal and interglobular domain processing of cartilage aggrecan in late stage cartilage degradation. *Matrix Biol* 2002; 21: 271-288.
- [28] Dean DD and Woessner J. Extracts of human articular cartilage contain an inhibitor of tissue metalloproteinases. *Biochem J* 1984; 218: 277-280.
- [29] Park SR, Jang KW, Park SH, Cho HS, Jin CZ, Choi MJ, Chung SI and Min BH. The effect of sonication on simulated osteoarthritis. Part I: effects of 1 MHz ultrasound on uptake of hyaluronan into the rabbit synovium. *Ultrasound Med Biol* 2005; 31: 1551-1558.
- [30] Korb AS, Shellock FG, Cohen MS and Bystritsky A. Low-Intensity Focused Ultrasound Pulsation Device Used During Magnetic Resonance Imaging: Evaluation of Magnetic Resonance Imaging-Related Heating at 3 Tesla/128 MHz. *Neuromodulation* 2014; 17: 236-241.