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ORIGINAL RESEARCH

Acupotomy Ameliorates Articular Cartilage Fibrosis in a Rabbit Model of Knee Osteoarthritis via the TGF-β1/Smad Pathway

Longfei Xing[1,*], Wenting Zhu[2,*], Xilin Chen[3,4], Rui Che[5], Jiangong Wu[1], Yan Guo[6], Chuxi Liang[1]

[1]Department of Traditional Chinese Medicine, Peking University People's Hospital, Beijing, People's Republic of China; [2]Department of Rehabilitation Medicine, The Third Affiliated Hospital of Southern Medical University, Southern Medical University, Guangzhou, People's Republic of China; [3]Department of Acupuncture and Rehabilitation, The Fifth College of Clinical Medicine, Guangzhou University of Traditional Chinese Medicine, Guangzhou, People's Republic of China; [4]Department of Acupuncture and Rehabilitation, Guangdong Second Hospital of Traditional Chinese Medicine, Guangzhou, People's Republic of China; [5]Department of Acupuncture and Physiotherapy, Beijing Water Resources Hospital, Beijing, People's Republic of China; [6]Beijing Hospital of Traditional Chinese Medicine, Capital Medical University, Beijing, People's Republic of China

*These authors contributed equally to this work

Correspondence: Chuxi Liang, Department of Traditional Chinese Medicine, Peking University People's Hospital, Beijing, 100029, People's Republic of China, Tel +86-15120058248, Email liangchuxi@126.com

Objective: This study aimed to investigate whether acupotomy can regulate the activity of factors related to the TGF-β1/Smad pathway, thereby reducing the degree of cartilage fibrosis in knee osteoarthritis (KOA) and alleviating cartilage degradation.

Methods: A modified Videman's long-term knee joint immobilization method in the extended position was used to establish the KOA model. Behavioral Lequesne MG scoring was performed before and after the acupotomy intervention. Masson staining was used to observe the morphology and structure of the cartilage using light microscopy and scanning electron microscopy (SEM). Western blotting and real-time quantitative polymerase chain reaction (RT-PCR) were used to detect the protein levels and mRNA expression of Smad3, Smad7, TGF-β1, collagen type I (Col-I), collagen type II (Col-II), and collagen type III (Col-III) in the cartilage tissue of acupotomy-treated KOA rabbits.

Results: The modified Lequesne MG score was significantly higher in the KOA group than that in the control group. Histologically, the cartilage in the KOA group exhibited fissures, matrix dissolution, reduced Col-II content, and obvious fibrosis. TGF-β1 and Smad3 in the TGF-β1/SMAD pathway were significantly upregulated, and Smad7 was down-regulated. Acupotomy treatment reduced the protein and mRNA expression levels of Col-I and Col-III, attenuated the loss of Col-II in cartilage extracellular matrix, and effectively delayed cartilage degeneration in KOA rabbits. This process may be achieved by regulating the factors related to the TGF-β1/SMAD pathway.

Conclusion: In the KOA rabbit model, acupotomy alleviated the degree of fibrosis by regulating factors related to the TGF-β1/Smad pathway, thereby delaying cartilage degeneration.

Keywords: cartilage, fibrosis, TGF-β1/Smad pathway, acupotomy, knee osteoarthritis

Introduction

Knee osteoarthritis(KOA) is caused by degenerative pathological changes including joint capsule thickening and degeneration, secondary osteophytes, synovial inflammatory lesions, cartilage degeneration, and subchondral bone sclerosis. Its incidence is expected to increase to 74.9% by 2050.[1,2] The main pathological characteristic of KOA is the degeneration of the articular cartilage. The formation and function of articular cartilage are affected by mechanical forces.[3] Studies have shown that abnormal mechanical stress can cause degenerative changes in the cartilage through oxidative stress, apoptosis, autophagy, ferroptosis.[4–6] An imbalance of mechanical stress in the knee joint is often considered an important pathogenic factor in the occurrence and development of KOA. Therefore, it is crucial to study how mechanical stimulation affects chondrocytes to better understand the pathophysiology of the disease and to develop effective clinical interventions.

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Articular cartilage fibrosis is an important pathological feature of KOA. Articular cartilage is an avascular connective tissue that relies on oxygen and nutrient diffusion from synovial fluid.[7] Under healthy conditions, chondrocytes primarily maintain metabolic homeostasis through glycolysis, with only 25% being dependent on oxidative phosphorylation.[8] However, injury or abnormal loading of the knee joint can disrupt this balance, causing immunometabolic shifts, dysregulation of the glycolytic cascade, lactate build-up, and acidification of the local microenvironment. These changes disrupt extracellular matrix(ECM) production, impair ATP synthesis, limit cell viability and survival, and trigger or exacerbate the fibrotic process.[9] Articular cartilage fibrosis is closely associated with an imbalance in the collagen network. Fibrosis is known to occur in KOA, including reduced collagen type II (Col-II) and increased Col-I levels, thereby compromising the structural integrity of the cartilage.[10,11] Additionally, collagen type III (Col-III), which is present in the form of finer fibers, interacts synergistically with other collagen types,such as Col-I, to provide essential structural support and mechanical strength, and is involved in the fibrotic pathological process of cartilage.[12] During fibrosis, accumulated and crosslinked collagens reduce ECM flexibility, thereby impeding joint mobility, whereas disordered proteoglycans affect water retention and shock absorption, impairing the normal biomechanical properties of the cartilage.[13] Concurrently, a thickened ECM and enhanced collagen deposition in fibrotic tissues can impede oxygen delivery, leading to tissue hypoxia, which impairs tissue homeostasis and cellular function.[14] Further studies have indicated that fibrosis can affect neurosensitization and inflammation, and is associated with pain and mobility, thereby affecting the degree of cartilage degeneration and exacerbating the pathological process of KOA.[15,16]

The TGF-β/Smad signaling pathway plays a pivotal role in cell biology and disease progression, primarily by regulating processes such as cell proliferation, growth, differentiation, apoptosis, and ECM remodeling.[17–19] In recent years, research has highlighted its role in fibrotic diseases.[20] Mechanistically, TGF-β activation promotes the synthesis and deposition of ECM components, including collagen and fibronectin, through Smad2/3-mediated signaling.[21] This regulatory function is particularly relevant for tissue repair and fibrosis. Moreover, biomechanical factors influence the TGF-β/Smad signaling pathway. A study by Jihee Kim[22] found that keloids, a common cutaneous fibrotic disorder, are characterized by increased local mechanical tension along with significant upregulation of TGF-β and Col-I, suggesting that the TGF-β/Smad pathway can be activated by mechanical stimulation.

Acupotomy is a therapeutic approach that combines acupuncture with surgical knife techniques. It alleviates joint pain and improves mobility by relaxing the soft tissues surrounding the knee joint, including the muscles, tendons, and ligaments, thereby regulating the abnormal mechanical stress in the knee. Our previous studies have demonstrated that acupotomy therapy of "Tendon-Regulation for Bone-Repair"can enhance the biomechanical properties of peri-knee soft tissues and delay cartilage degeneration in the knee.[23–25] However, it remains unclear whether the mechanical effects of acupotomy can regulate fibrotic changes in the KOA cartilage. Therefore, this study focused on cartilage fibrosis and aimed to investigate whether the mechanical effects of acupotomy can effectively delay fibrotic changes in KOA cartilage through the TGF-β/Smad signaling pathway, thereby exerting a protective effect on cartilage.

Materials and Methods

Animal Species and Grouping

Eighteen healthy clean grade male New Zealand rabbits, 6 months old, weighing 2.5±0.5 kg, were purchased from Beijing Fulong Tengfei Experimental Animal Research Institute Ltd., Beijing, China, (Certificate No: SYXK2018–0041) and housed in the laboratory of the same institute. The housing environment was 12/12h light/dark, single-cage housing, free feeding and drinking, constant temperature of 23±1 °C, and relative humidity of 50%. After one week of acclimatization, six rabbits were randomly selected as the control group (control group) using a random number table method. The remaining rabbits were used to establish the KOA model using the modified Videman method (left hindlimb immobilization in the extended position). After modeling, the rabbits were randomly divided into a model group (KOA group) and an acupotomy group (acupotomy group), with 6 rabbits in each group. All experimental procedures were performed in strict accordance with the Guiding Opinions on the Humane Treatment of Laboratory Animals issued by the Ministry of Science and Technology of the People's Republic of China in September 2006, and strictly followed the

requirements of ethical review. The ethical review institution was the Institutional Animal Care and Use Committee of the Peking University People's Hospital, approval number: 2020pHE100).

Model Establishment and Evaluation

After one week of acclimatization, the KOA rabbit model was established using the modified Videman method. After 12 h of fasting, the rabbits were anesthetized via marginal ear vein injection with 3% sodium pentobarbital solution (Bioway, Beijing, China) at a dose of 30 mg/kg. If the corneal reflex is significantly weakened or absent, and the skin is clamped with hemostatic forceps without pain, the anesthesia effect is appropriate. During anesthesia, add 2–3 drops of lubricant to the rabbits' eyes every 15 min to prevent the rabbits' eyes from drying out. The rabbits were fixed on the operating table and the hair on the left hind limb was shaved. With the left hindlimb maintained in the extended position, double-sided foam tape was wrapped around the limb from the inguinal region to below the ankle joint. Absorbent cotton was placed at the root of the inguinal region to prevent abrasion and resin bandages were wrapped around the surface of the foam tape under moderate tension. A suitable length of the polymer bandage was rapidly wrapped around the resin bandage for reinforcement, followed by an anti-chew bandage before the polymer bandage was dried. To monitor the blood supply to the limb extremities, the toes of the rabbit and part of the dorsum of the foot were left exposed. If slight swelling is observed, the immobilization device should be adjusted appropriately; in cases of significant swelling, ulcers, tissue necrosis, or other severe symptoms, the device should be removed immediately. After the swelling subsided, the limb was re-immobilized with continuous immobilization for 4 weeks. After four weeks, the Lequesne MG behavioral scoring system was used as the model evaluation criterion.

Intervention Methods

Control Group

The rabbits were raised under the same conditions without any therapeutic intervention and were grabbed identically during the intervention periods of the other groups.

KOA Group

The Interventions Were the Same as Those in the Control Group.

Acupotomy Group

The acupotomy intervention was performed after successful modeling. The rabbits were immobilized and the skin over the affected knee joint was shaved to expose the intervention sites. The treatment points for acupotomy were selected as tendon insertions of the vastus medialis, vastus lateralis, rectus femoris, and biceps femoris muscles as well as 1–2 myofascial trigger points around the knee joint. Disposable acupotomy (0.3 mm×25 mm) was used for acupotomy treatment following the four-step procedure. The intervention was conducted once a week for 4 consecutive weeks.

Behavioral Evaluation

The Lequesne MG behavioral scoring system was used, and before and after the intervention, the modified Lequesne MG knee osteoarthritis scoring scale was used for behavioral evaluation in each group. The assessment included knee pain response to stimulation, gait changes, ROM, and swelling of the knee joint. The knee joint score of normal rabbits was 0 points,1–4 points indicated mild KOA, 5–8 points indicated moderate KOA, and a score above 8 indicated severe KOA.

Specimen Collection

After behavioral evaluation, all rabbits were sacrificed rabbits were sacrificed by combining deep anesthesia and air embolization in strict accordance with aseptic operation. And the left knee joint was surgically opened to fully expose the joint cavity. Sufficient cartilage tissue was carefully and accurately scraped using a sterile scalpel, ensuring the integrity and accuracy of the collected tissue. The collected cartilage tissue was immediately placed in pre-labeled cryovials, articular cartilage samples used for Western Blot and RT-PCR then rapidly transferred to a liquid nitrogen tank, and finally stored at −80°C to maximize the preservation of protein activity and stability.

Scanning Electron Microscopy (SEM) of Articular Cartilage

The harvested bone-subchondral bone complexes were rinsed with normal saline and then washed by phosphate-buffered saline (PBS) 3times, for 3 min each. The specimens were trimmed into tissue blocks of 3×3×5 mm in glutaraldehyde-polyformaldehyde solution and then fixed in the same solution for 24 h. The fixed specimens were rinsed in 0.1 mol /L sodium cacodylate buffer, followed by post-fixation in 1% osmium tetroxide for 2 h. After rinsing, the specimens were subjected to gradient dehydration with ethanol and transferred to isoamyl acetate for overnight storage. After removal, the samples were dried using a carbon dioxide critical point dryer, adhered to specimen stubs, sputter-coated with gold for electrical conductivity under vacuum, and observed and photographed under a scanning electron microscope (SEM).

Masson Staining of Articular Cartilage

Articular cartilage tissues were fixed in 10% neutral buffered formalin, followed by routine dehydration and paraffin embedding. Serial sections of 3 μm thickness were cut, routinely dewaxed in water, mordanted at room temperature for 8–12 hours, and then rinsed three times for 3 min each. Subsequently, the sections were dripped and stained with celestine blue solution and Mayer's hematoxylin solution for 2–3 minutes each, and washed twice with distilled water for 10–15 seconds each time. After differentiation in an acid differentiation solution for several seconds and rinsing with water, the sections were stained with ponceau-fuchsin solution for 10 min, washed twice with distilled water, treated with phosphomolybdic acid solution, and immediately stained with aniline blue solution for 5 min. Excess stain was washed with a weak acid solution, and the sections were treated with a covering reagent for 2 min, dehydrated, cleared twice with xylene, and mounted with neutral balsam. Images were captured using a light microscope.

Western Blot

Articular cartilage samples were retrieved from a refrigerator at-80 °C for grinding and then collected in centrifuge tubes. A lysis working solution was prepared by mixing RIPA lysis buffer (Servicebio, Wuhan, China) with PMSF (Servicebio) at a ratio of 100:1 and was added to the centrifuge tubes for total protein extraction. The protein concentration was quantified using a BCA protein assay kit (Solarbio, Wuhan, China). Electrophoresis was carried out on 12% SDS-polyacrylamide gel electrophoresis (SDS-PAGE) gels at 80 V for 2 h, transferred to 0.22 μm PVDF membranes (Millipore, Boston, MA, USA), and processed at 100 V for 30 min. Subsequently, the PVDF membranes were placed in a quick blocking solution (Solarbio, Beijing, China). The membranes were then incubated overnight at 4 °C in a primary antibody dilution buffer. The following primary antibodies were used: Col-I (1:1000), Col-II (1:2000), Col-III (1:5000), TGF-β1 (1:1000), Smad-3 (1:2000), Smad-7 (1:5000), and β-actin (1:10,000). The following day, the membranes were incubated with horseradish peroxidase (HRP)-conjugated secondary antibodies for 1 h at room temperature and the protein bands were visualized using an ultrasensitive ECL chemiluminescence kit. The imaging system software used was Image Lab™ Touch Software (Version 2.4.0.03) and the imager model was ChemiDoc™ MP. Chemiluminescence blotting was performed for imaging. During the chemiluminescence process, the optimal automatic exposure mode was prioritized. If this mode could not display the mark, the manual exposure mode was selected. The gray values of the protein bands were analyzed using the ImageJ software (Rockville, MD, USA).

Real-Time Quantitative Polymerase Chain Reaction (RT-PCR) Analysis

Articular cartilage samples were obtained using the same method as that used for Western blotting. Total RNA was homogenized using RNA extraction reagent (Servicebio, Wuhan, China). RNA was reverse-transcribed into cDNA, according to the manufacturer's instructions. Real-time quantitative polymerase chain reaction amplification of cDNA was performed using the SYBR Green qPCR Master Mix (Servicebio, Wuhan, China). Specific PCR primers for glyceraldehyde-3-phosphate dehydrogenase (GAPDH), Col-I, Col-II, Col-III, TGF-β1, Smad-3, and Smad-7 were designed based on previously published sequences. The rt-PCR reaction conditions were as follows: initial denaturation at 95°C for 5 min, followed by 45 cycles of denaturation at 95°C for 30s, annealing at 60°C for 30s, and extension at 72°C for 30s. The relative expression levels of the target genes were calculated using the 2^(− ΔΔCt) method and were normalized to the mRNA expression level of GAPDH. Sequences of the forward and reverse primers are listed in Table 1.

Table 1 Primers Used in This Study

Gene	Primer Sequences
TGF-β1	Forward: 5′-CTGCTGTGGCTCCTAGTGTTGA-3′
	Reverse: 5′-AGCCGCAGTTTGGACAGGAT-3′
Smad-3	Forward: 5′-GCTCCCTCATGTCATCTACTGCC-3′
	Reverse: 5′-GATTCACGCAAACCTCATCCTTC-3′
Smad-7	Forward: 5′-AGAAAGTGCGGAGCAAAATCG-3′
	Reverse: 5′-TGCATGAACTCGTGGTCGTT-3′
Col-I	Forward: 5′-TGGTGAATCTGGACGTGAGGG-3′
	Reverse: 5′-TTATGCCTCTGTCGCCCTGTT-3′
Col-II	Forward: 5′-AGCGGTGACTACTGGATAGA-3′
	Reverse: 5′-CTGCTCCACCAGTTCTTCTT-3′
Col-III	Forward: 5′-GTACATCTGGTCATCCTGGCTC-3′
	Reverse: 5′-CTTCCAGGTCGTCCTGATTCTC-3′
GAPDH	Forward: 5′-TGAAGGTCGGAGTGAACGGAT-3′
	Reverse: 5′-CGTTCTCAGCCTTGACCGTG-3′

Statistical Analysis

All data were analyzed using SPSS 20.0, and expressed as the mean ±standard deviation ($\overline{x}$±s). For data that conformed to a normal distribution and homogeneity of variance, one-way analysis of variance (ANOVA) was performed, and the least significant difference (LSD) test was used for pairwise comparisons between groups. If the data did not conform to the normal distribution and homogeneity of variance simultaneously, a nonparametric test (Mann–Whitney *U*-test) was applied. Statistical significance was set at α=0.05 as statistical significance was set at $P<0.05$.

Results

Acupotomy Reduces Osteoarthritis Scores

After modeling (before intervention), compared with the control group, the Lequesne MG scores of the KOA and acupotomy groups were significantly higher ($P<0.01$, $P<0.05$, respectively). However, there were no statistically significant differences in the Lequesne MG scores between the KOA and acupotomy groups (both $P>0.05$), indicating that after 4 weeks of left hindlimb immobilization in the extended position with the modified Videman method, rabbits in both groups exhibited obvious behavioral changes, such as movement disorders and lameness of the impaired limb, suggesting successful model establishment, consistent baselines, and comparability between groups. After intervention, compared with the control group, the Lequesne MG score of the KOA group was significantly increased ($P<0.01$); compared with the KOA group, the Acupotomy group showed a notable decreasing trend in Lequesne MG scores ($P>0.05$), which demonstrated that acupotomy treatment can improve the behavioral performance of KOA rabbits (Figure 1).

Acupotomy Improves Morphological Damage of Cartilage in KOA Rabbits

Masson staining was used to observe the articular cartilage and collagen fibers. Usually, collagen fibers and cartilage are stained blue, cytoplasm and fibrin red, and cell nuclei are bluish-black. In the control group, the four-layer cartilage structure was distinguishable with a clear tidemark. The KOA group exhibited rough cartilage surfaces with fissures, loss of staining, obvious fibrosis, and disorganized, locally fractured, and degraded collagen fibers. Many flame-shaped dark red-stained protrusions were observed near the tidemark, which were relatively high and widespread. Damage to the articular cartilage surface was clear. Compared to the KOA group, the acupotomy group showed reduced loss of cartilage staining, more orderly arranged fibers, significantly diminished fibrosis, and an intact tidemark (Figure 2).

SEM was used to observe the cartilage matrix and collagen fibers. In the control group, the cartilage matrix was homogeneous without exposure of the subchondral bone, and the collagen fibers were uniform in size and arranged in a loose and continuous network with a small number of protein particles attached to their surfaces. The KOA group showed extensive dissolution of the cartilage matrix, with a certain degree of depression. The collagen fibers were

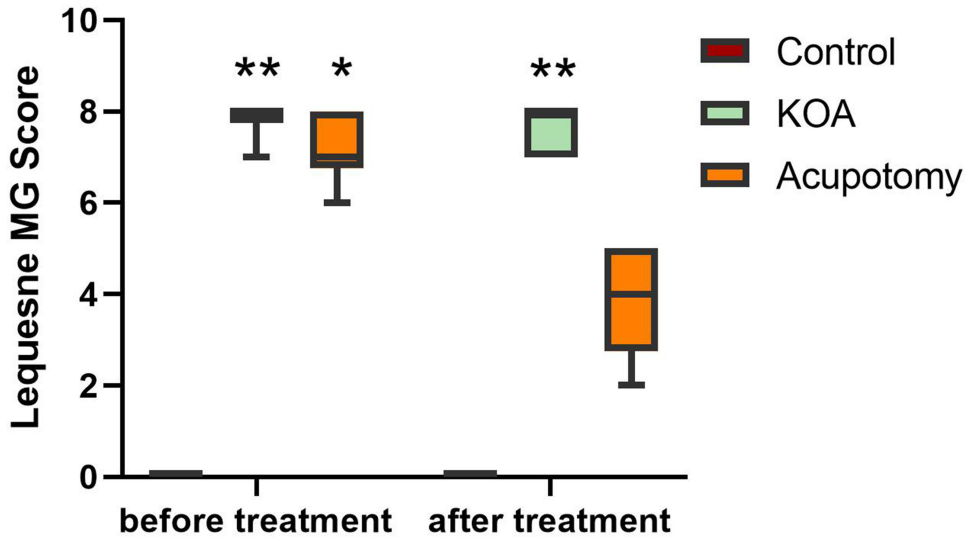

Figure 1 Effects of Acupotomy on Lequesne MG Scores of the KOA rabbits. Values are expressed as median (interquartile range)/M (IQR); N=6. $^{**}P < 0.01$, $^{*}P < 0.05$, vs Control group.

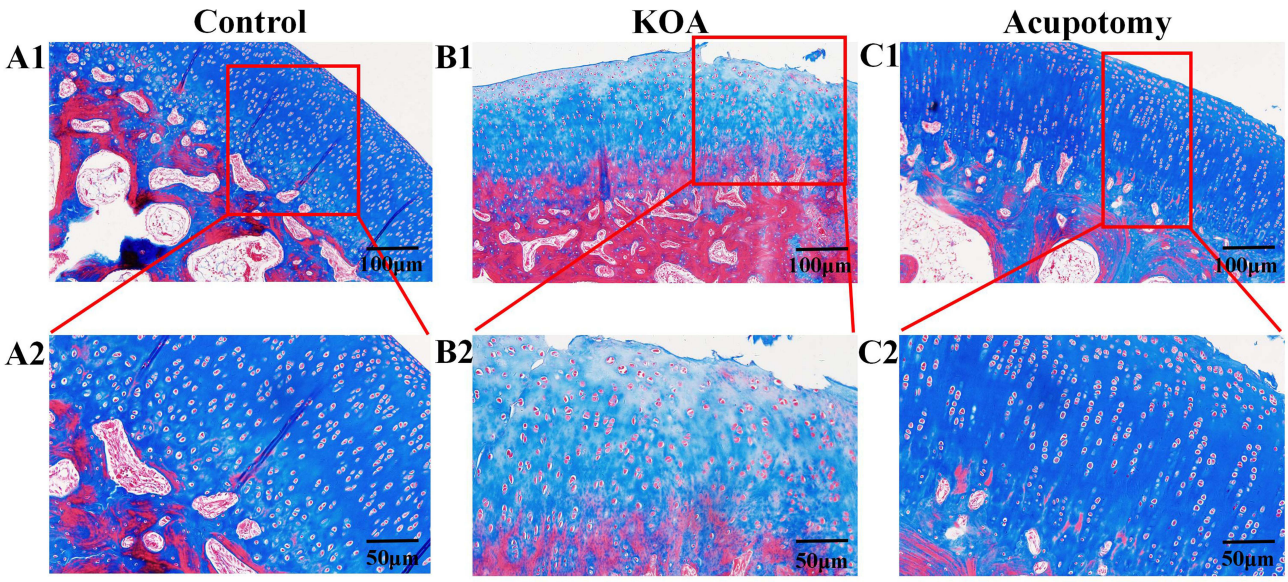

Figure 2 Effects of acupotomy on cartilage fibrosis morphology in KOA rabbits as observed by Masson staining. (**A1**–**C1**): Bars 100μm; (**A2**–**C2**): Bars 50μm.

significantly thickened in parts, accompanied by massive dissolution, fracture, uneven thickness, and disorganized arrangement. A small amount of protein particles was attached to the fiber surfaces with uneven sizes. In the Acupotomy group, the cartilage matrix was dissolved in a small area, collagen fibers were slightly thickened and partially dissolved, and a large number of protein particles were attached to the surfaces (Figure 3).

Acupotomy Reduces Cartilage Matrix Damage and Alleviates Fibrosis

Western blotting and RT-PCR were used to detect the protein and gene expression levels of Col-I, Col-II, and Col-III to evaluate the degree of cartilage fibrosis. Compared to the control group, the KOA group exhibited abnormally high expression of Col-I and Col-III proteins ($P<0.01$), whereas Col-II protein expression was abnormally low ($P<0.01$). Compared with the KOA group, the acupotomy group showed a significant decrease in Col-I protein content ($P<0.05$), a marked reduction in Col-III protein content ($P<0.01$), and a significant increase in Col-II protein content ($P< 0.01$).

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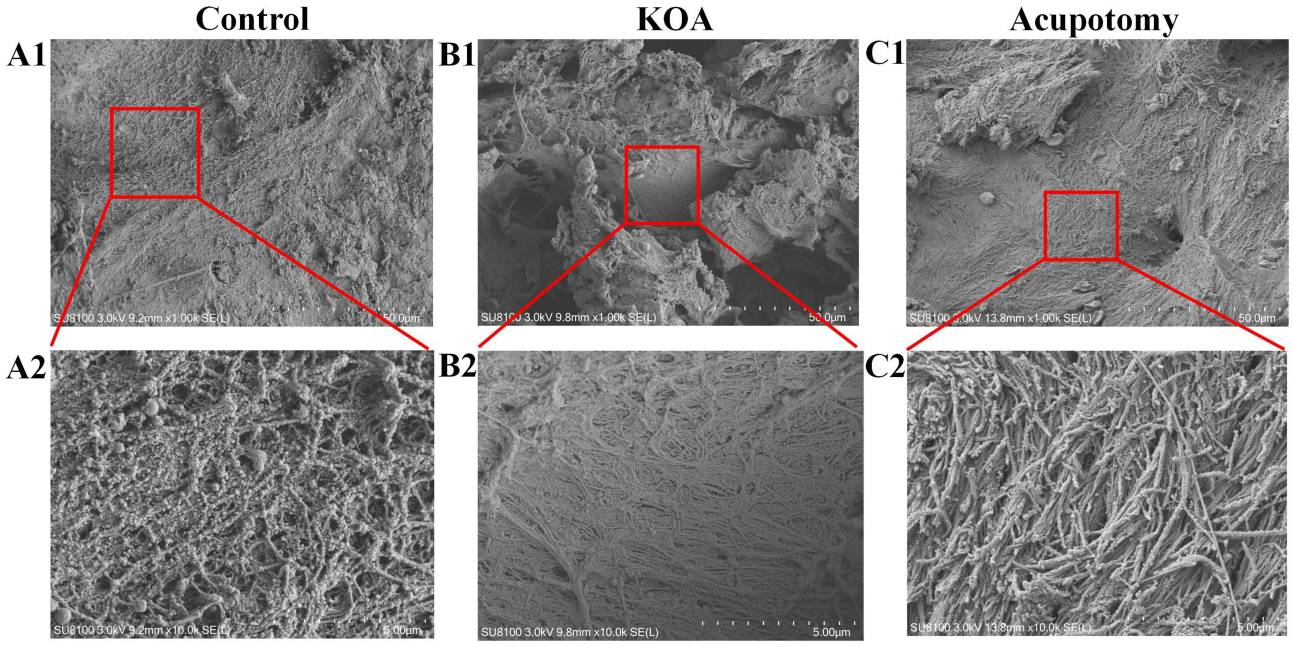

Figure 3 Effects of acupotomy on the ultrastructure of cartilage fibrosis in KOA rabbits as observed by SEM. (**A1**–**C1**) Bars 50μm; (**A2**–**C2**) Bars 5μm.

Compared to the control group, the KOA group exhibited abnormally high expression of Col-I and Col-III mRNA (P<0.01), whereas Col-II mRNA expression was abnormally low (P<0.01). Compared with the KOA group, the acupotomy group showed a significant decrease in both Col-I and Col-III mRNA expression (P<0.05) and a marked increase in Col-II mRNA expression (P<0.01). These results indicate that acupotomy therapy can regulate the synthesis and deposition of different types of collagen and alleviate cartilage fibrosis (Figure 4).

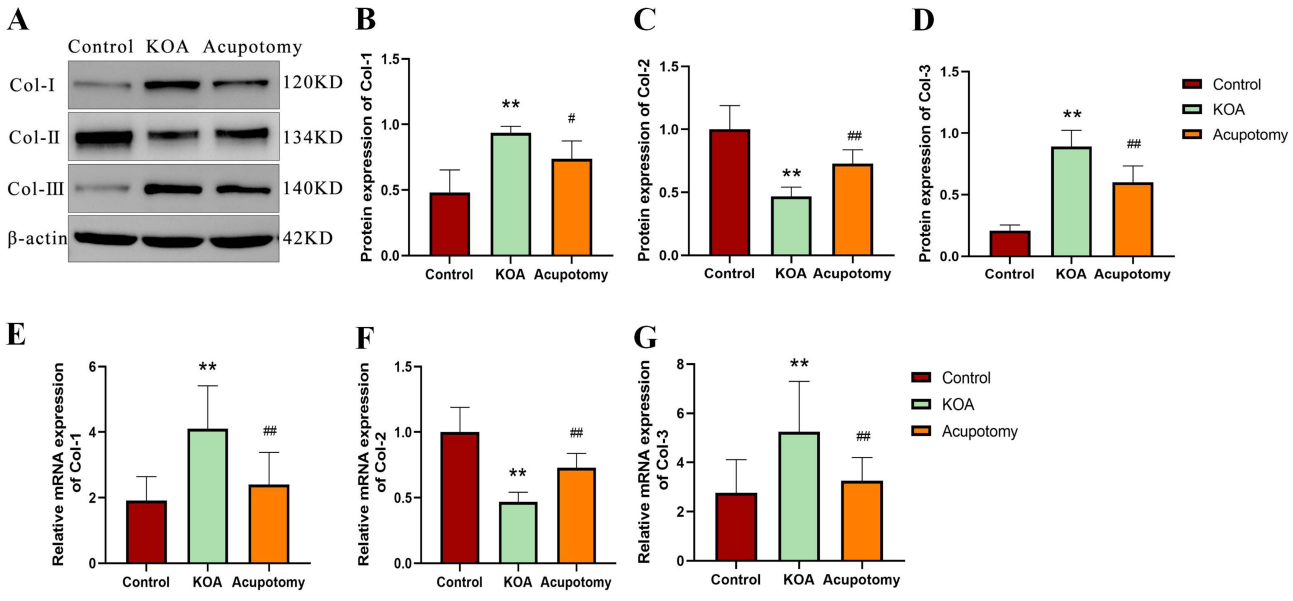

Figure 4 Effects of acupotomy on cartilage collagen proteins in KOA rabbits. (**A**) Western Blot images of Col-I, Col-II, and Col-III; (**B**–**D**) Comparisons of Col-I, Col-II, and Col-III protein expressions among groups; (**E**–**G**) Comparisons of Col-I, Col-II, and Col-III gene expressions among groups. Compared with Control group:$^{**}P$ < 0.01; compared with KOA group:$^{\#\#}P$ < 0.01, $^{\#}P$ < 0.05.

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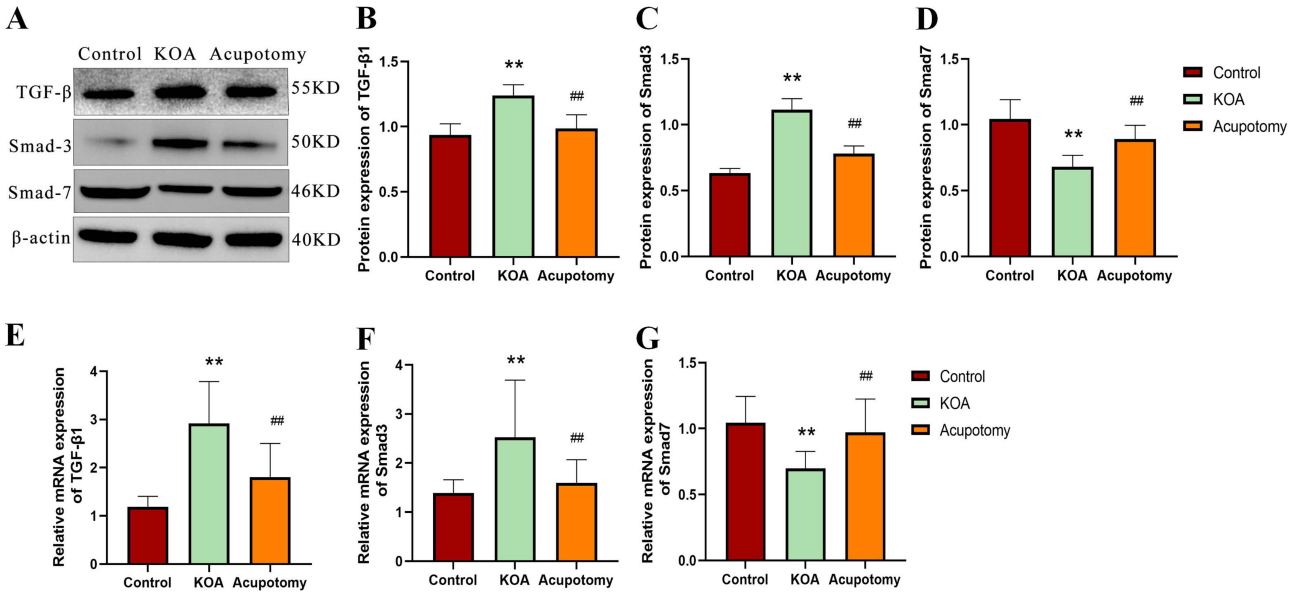

Figure 5 Effects of acupotomy on the TGF-β1/Smad signaling pathway in the cartilage of KOA rabbits. (**A**) Western Blot images of TGF-β1, Smad3, and Smad7; (**B–D**) Comparisons of TGF-β1, Smad3, and Smad7 protein expressions among groups; (**E–G**) Comparisons of TGF-β1, Smad3, and Smad7 gene expressions among groups. Compared with Control group: $^{**}P < 0.01$; compared with KOA group: $^{\#\#}P < 0.01$.

Acupotomy Regulates the Expression of Proteins and Genes Related to the TGF-β1/Smad Signaling Pathway in Cartilage

Compared with the control group, the KOA group exhibited a significant increase in the protein content of TGF-β1 and Smad3 ($P<0.01$) and a marked decrease in Smad-7 protein content ($P<0.01$). In contrast, compared to the KOA group, the acupotomy group showed significant downregulation of TGF-β1 and Smad3 protein expression ($P<0.01$) and significant upregulation of Smad-7 protein content ($P< 0.01$).

Compared with the control group, the KOA group demonstrated a significant increase in the mRNA levels of TGF-β1 and Smad3 ($P<0.05$) and a significant reduction in Smad-7 mRNA levels ($P<0.05$). Conversely, compared to the KOA group, the acupotomy group exhibited significant downregulation of TGF-β1 and Smad-3 mRNA levels ($P<0.05$) and significant upregulation of Smad-7 mRNA levels ($P<0.05$). These findings indicate that acupotomy therapy can alleviate cartilage fibrosis and delay cartilage degeneration by regulating the expression of proteins and genes related to the TGF-β1/SMAD signaling pathway (Figure 5).

Discussion

The results of this study demonstrated that acupotomy can reduce the Lequesne MG score in KOA rabbit models, effectively decrease the deposition of Col-I and Col-III, inhibit the degradation of Col-II, mitigate cartilage collagen degradation, and alleviate cartilage fibrotic changes. These findings suggest that acupotomy can improve behavioral outcomes such as pain relief and joint mobility in KOA model rabbits by attenuating cartilage fibrotic changes, thereby delaying cartilage degeneration. Furthermore, our study revealed that the inhibitory effect of acupotomy on cartilage fibrosis is mediated by regulation of the TGF-β1/Smad signaling pathway.

KOA is one of the most prevalent joint diseases and is characterized by pathological changes including cartilage degeneration, reduced anabolism, subchondral bone remodeling, synovial inflammation, and osteophyte formation, leading to joint pain and limited mobility. Cartilage fibrosis is a pathological feature of KOA.[26] Once cartilage is damaged, abnormal chondrocyte proliferation occurs. Eventually, proliferating chondrocytes transform into a fibroblast-like phenotype by synthesizing Col-I and inducing degradation and dedifferentiation of adjacent hyaline cartilage, forming a thick layer of fibrocartilage-like tissue.[27,28] These changes result in stiffer cartilage, reducing its biomechanical properties, making it susceptible to degradation by external forces, and ultimately altering the articular cartilage

phenotype, thereby exacerbating KOA.[29] Liu Q[30] demonstrated that upregulation of DDX5 can downregulate Col-I expression and upregulate Col-II expression, thereby inhibiting cartilage fibrosis and degradation, and delaying the progression of KOA. Similarly, Kuang[31] found that panaxatriol exerts anti-senescence effects by targeting UFL1, alleviating cartilage repair fibrosis, and thereby protecting cartilage. Our previous studies have shown that acupotomy can effectively delay cartilage degeneration by releasing periarticular soft tissue. Through Masson staining and SEM, this study further revealed that acupotomy can alleviate cartilage fibrosis, thereby playing a protective role in the cartilage.

Fibrosis occurs in various tissues, and articular cartilage fibrosis also develops in OA, playing a key role in pathological progression and cartilage destruction in OA.[32] Knee joint cartilage is a type of hyaline cartilage whose smooth surface has a lubricating effect and can absorb external forces to protect bone.[7] Although chondrocytes are quiescent cells with low proliferative capacity, they exhibit highly active protein synthesis abilities.[33] The chondrocyte ECM contains various types of collagen secreted by chondrocytes. Their secretion levels vary with changes in the intra-articular environment and cartilage quality is maintained through a balance between the degradation and synthesis of different collagens. In healthy articular cartilage, Col-I accounts for only 1.7% of the total collagen area, whereas Col-II accounts for nearly 100%.[34] Biochemical analyses have also shown that 0.2% of the total collagen in articular cartilage is Col-I and 96% is Col-II.[35] Reduced and increased expressions of Col-II and Col-I, respectively, are key characteristics of articular cartilage fibrosis. During KOA progression, proliferative chondrocytes transport or dedifferentiate into a fibroblast-like phenotype and undergo significant changes in cell shape, metabolism, and cytoskeletal structure. This ultimately leads to increased synthesis of Col-I, cessation of aggrecan and Col-II expression, and formation of difficult-to-repair fibrocartilage-like tissue, thereby disrupting the cartilage structure.[36] Col-III is present at low levels in the normal articular cartilage; however, its expression may increase during cartilage degeneration or injury. Studies have shown that Col-III expression is up-regulated during cartilage fibrosis.[37] Consistent with these findings, our study found that the protein and mRNA expression levels of Col-I and Col-III increased, whereas those of Col-II decreased in the KOA group. In contrast, acupotomy promoted the protein and mRNA expression of Col-II and inhibited the expression of Col-I and Col-III, thereby delaying the progression of cartilage fibrosis in KOA patients.

Fibrosis is a pathological process that involves the excessive accumulation of collagen and other ECM components in tissues, leading to tissue stiffening and functional impairment.[38] The TGF-β1/SMAD pathway is a key pathogenic mechanism in tissue fibrosis.[39] TGF-β1 is a multifunctional cytokine that plays a fundamental role in regulating cellular processes and ECM components, including collagen, fibronectin, and elastin, and serves as a key mediator in the development of tissue fibrosis and inflammation.[40] TGF-β1 exerts its biological effects by activating downstream mediators, including Smad-2 and Smad-3, and is negatively regulated by Smad-7 expression.[40] Smad-3 is a core component of the TGF-β1 signaling pathway and plays a crucial role in intracellular signal transduction and regulation of gene expression.[41] The role of Smad-3 plays a critical role in the development of fibrosis.[42] Activated by TGF-β signaling, Smad-3 promotes fibroblast activation and differentiation, increases the synthesis of collagen and other matrix proteins, and drives the fibrotic process. Smad-3 can also enhance cellular responsiveness to TGF-β by forming a positive feedback loop. This implies that, once the fibrotic process is initiated, it may be self-sustaining or even exacerbated. Smad-7 is a member of the Smad protein family, which acts as an inhibitory factor that binds to TGF-β1 receptors to prevent the activation of Smad-2 and Smad-3, thereby suppressing the activation of the TGF-β1 signaling pathway.[43,44] In this study, we found that the protein and mRNA expression levels of TGF-β1 and Smad-3 were upregulated, whereas those of Smad-7 were downregulated in the articular cartilage of the KOA group. In contrast, compared to the KOA group, the protein and mRNA expression levels of TGF-β1 and Smad3 were downregulated, and those of Smad-7 were upregulated in the acupotomy group. Combined with the morphological results and the changes in the protein and mRNA expression of Col-I, Col-III, and Col-II in cartilage, these findings confirm that acupotomy can inhibit the progression of cartilage fibrosis by regulating the TGF-β1/Smad signaling pathway, thereby exerting a protective effect on cartilage and delaying the progression of KOA.

This study has certain limitations. To reduce the influence of surgical factors on the results, the same person performed the acupotomy in this study. However, because the acupotomy operation was conducted under non-visualized conditions (without direct exposure of the lesion site), differences in the acupotomy operation could not be completely avoided. Therefore, it is necessary to use ultrasound-guided technology for a more accurate operation in

future studies. In addition, knee osteoarthritis is divided into different pathological stages, and our team still needs to conduct a comparative study on KOA at different pathological stages to clarify the best intervention timing and treatment plan for acupotomy.

Conclusion

In summary, this study showed that acupotomy can play a protective role in cartilage by regulating the TGF-β1/SMAD pathway, reducing cartilage matrix degradation, and inhibiting cartilage fibrosis in KOA patients. As a complementary and alternative therapy, acupotomy may provide new treatment ideas and methods for patients with KOA.

Abbreviations

KOA, knee osteoarthritis; Col-I,collagen type I; Col-II,collagen type II; Col-III,collagen type III; SEM,scanning electron microscopy; RT-PCR,real-time quantitative polymerase chain reaction; PBS,phosphate-buffered saline; SEM,scanning electron microscope; GAPDH,glyceraldehyde-3-phosphate dehydrogenase;ECM, extracellular matrix.

Ethics Approval

All animal experiments were performed at Peking University (Beijing, China) according to the guidelines of the China Council on Animal Care and Use. This study was approved by the Institutional Animal Care and Use Committee of the Peking University People's Hospital (approval number: 2020pHE100). Every effort was made to minimize pain and discomfort in the animals.

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Disclosure

The authors report no conflicts of interest in this work.

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