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ORIGINAL RESEARCH

# RETRACTED ARTICLE: Acupotomy Improves Synovial Hypoxia, Synovitis and Angiogenesis in KOA Rabbits

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**Purpose:** Knee osteoarthritis (KOA) is a chronic inflammatory disease highly associated with intra-articular hypertension, hypoxia and angiogenesis of synovial tissue. Our previous studies showed that acupotomy could treat KOA in a variety of ways, including reducing cartilage deterioration and enhancing biomechanical qualities. However, the mechanism of hypoxia and angiogenesis induced by acupotomy in KOA synovium remains unclear. This study looked for the benign intervention of acupotomy in synovial pathology.

**Methods:** The rabbits were divided into 3 groups, Normal group, KOA group, and KOA + Acupotomy (Apo) group, with 11 rabbits in each group. The KOA rabbit model was established by the modified Videman method with six weeks. The KOA + Apo group performed the intervention. The tendon insertion of vastus medialis, vastus lateralis, rectus femoris, biceps femoris, and anserine bursa were selected as treatment points in rabbits. Rabbits were treated once every 7 days for 3 weeks. We observed the intra-articular pressure and oxygen partial pressure (BOLD MRI). Synovial morphology was monitored by Hematoxylin-Eosin Staining (HE Staining). The expression of hypoxia inducible transcription factor-1α (HIF-1α), vascular endothelial growth factor (VEGF), interleukin-1β (IL-1β) and tumour necrosis factor-α (TNF-α) was detected using Immunohistochemical (IHC), Western Blot and Enzyme-Linked Immunosorbent Assay (ELISA).

**Results:** Acupotomy reduced intra-articular hypertension and improved the synovial oxygen situation, synovial inflammatory and angiogenesis. HIF-1α, VEGF, IL-1β and TNF-α expression were downregulated by acupotomy.

**Conclusion:** Acupotomy may reduce inflammation and angiogenesis in KOA rabbit by reducing abnormally elevated intra-articular pressure and improving synovial oxygen environment. The above may provide a new theoretical foundation for acupotomy treatment of KOA.

**Keywords:** acupotomy, KOA, synovium, intra-articular pressure, hypoxia, angiogenesis

## Introduction

KOA accounts for about 85% of osteoarthritis (OA) cases worldwide, and its prevalence has more than doubled with the development of the aging population.[1,2] According to recent research, OA is an active dynamic change of intra-articular homeostasis caused by biomechanical abnormalities, involving complex inflammatory and metabolic factors.[3] The development of KOA implicated the cartilage, subchondral bone, meniscus, muscle, tendon, synovium, and infrapatellar fat pad (IFP).[4] The knee joint is a typical synovial joint. Synovium provides nutrition and oxygen for cartilage by producing synovial fluid, which plays a crucial role in the metabolism of cartilage.[5] Synovium is a tissue that usually becomes inflammatory, hyperplastic, angioplastic, and fibrotic due to its rich vascular and nerve distribution.[6] Along with cartilage degradation, the typical feature and the primary pathogenic factor of KOA are chronic low-grade inflammation

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of the synovium and extensive angiogenesis.[7,8] Synovitis correlates with the degree of pain and joint dysfunction and may occur before significant changes in cartilage promote faster cartilage destruction.[9] IL-1β and TNF-α are critical inflammatory mediators in the pathological process of synovitis.[10] Angiogenesis causes structural damage and pain by promoting the invasion of inflammatory cells and the growth of local pain receptors.[11] VEGF is an extremely specific angiogenic factor that directly contributes to KOA synovial angiogenesis.[12]

Hypoxia is a prominent feature of diseases such as arthritis, atherosclerosis, inflammatory bowel disease, infection, obesity and cancer.[13] Synovitis and angiogenesis may associate with changes in synovial microvascular oxygen system.[14] HIF-1α is the new therapeutic target for treating chronic and persistent diseases as a crucial component of the cellular response to hypoxia.[15] HIF-1α in synovium was positively correlated with the severity of KOA, which may be related to the role of increased HIF-1α in leading to persistent chronic synovitis and angiogenesis.[16] The non-invasive blood oxygen level-dependent magnetic resonance imaging (BOLD-MRI) is used to identify early hypoxia and changes of vascular joints by detecting the response of tissues to vascular stimulation.[17] Deoxyhemoglobin (DHb) is an endogenous contrast agent that is paramagnetic and positively correlated with changes in lateral relaxation rate (R2*), while the concentration of DHb is inversely proportional to the oxygen partial pressure of local blood flow.[18,19] Therefore, BOLD-MRI was used in measuring the oxygen partial pressure and blood flow of synovial tissue in this study.

Interestingly, the intra-articular pressure could influence the synovial oxygen environment.[20] The abnormally elevated intra-articular pressure in KOA will exceed the terminal perfusion pressure of synovium, resulting in synovium ischemia and reperfusion injury.[21] Insufficient perfusion caused by high pressure and increased metabolic demand of synovium in the joint cavity can lead to hypoxia in the inflammatory joint in the acute stage of arthritis.[22] Under hypoxia condition, the biochemical characteristics of synovium are changed, and the persistent inflammatory changes and compensatory angiogenesis in synovium may in turn contribute to cartilage injury, osteophyte formation and meniscus pathology,[23,24] which result in a vicious circle and aggravate the progress of KOA.

Acupotomy therapy is a minimally invasive therapy of traditional Chinese medicine, which combined both traditional acupuncture and modern surgical principles, and could dredge meridians and release tissue adhesion because of the specific bit tool of acupotomy could needle, cut, strip, and shovel.[25] Acupotomy has been shown to be beneficial in the treatment of KOA in clinical trials, and it is feasible, inexpensive, effective, and safe.[26] Biomechanical factors are currently recognized as the pathogenesis of KOA.[27] Our previous animal studies discovered that acupotomy could alleviate cartilage matrix degradation and repair cartilage injury after releasing adhesive ligament and extensor and flexor muscle attachment points.[28,29]

Therefore, this paper describes an investigation of the effect of acupotomy on intra-articular pressure, synovial oxygenation level, proinflammatory cytokines in the synovial fluid, and factors associated with synovial hypoxia and angiogenesis in the synovium to explore whether acupotomy relieves synovial inflammation and angiogenesis in KOA and whether it is associated with improving synovial hypoxia and reducing the activity of HIF-1α. As a result, this study shall provide a stronger theoretical basis for the effectiveness of acupotomy.



## Materials and Methods

### Animals and Study Design

Six-week-old healthy clean-grade New Zealand male rabbits were obtained from Beijing Fulong Tengfei Experimental Animal Research Institute Ltd (Beijing, China, Certificate Number: SYXK 2018-0041). The animals were maintained under the same feeding and environmental conditions. Housing environment: 12/12 h light/dark, single cage housing, free feeding and drinking, constant temperature 17–20°C, relative humidity 40%–60%. The experimental procedures carried out on animals were approved and reviewed by the Experimental Animal Ethics Sub-Committee, Academic Committee of Beijing University of Chinese Medicine (Approval number: BUCM-4-2022041502-2114). All animal experiments were conducted according to the Guidelines of the NIH for the welfare of laboratory animals.

The experiment was conducted, after the rabbits had been adaptively fed for 7 days. The animals were divided into Normal group, KOA group, and KOA + Apo group (11 rabbit/group), via random number table. And in each group 4 rabbits were used for BOLD MRI measurement, and the remaining 7 rabbits were used for other tests.

## Induction of KOA Rabbits

After 1 week of acclimatization, and 12 hours of fasting, the animals in KOA and KOA + Apo groups were KOA modeled with the modified Videman method.[28] Their left hind limbs were extended and immobilized. Three percent pentobarbital sodium solution was injected through the ear vein at 30mg/kg. Skin preparation in the inguinal to ankle area of the left hind limb. One experimenter pulled the rabbit's lower limb into a fully extended position, while the other one softened a resin bandage by soaking it in hot water at 65–85 °C, and fixed it from the groin to the ankle. The rabbit was kept with its knee joint extended at 180° and the ankle joint plantarflexed at 60°. The toes were left out to observe the blood supply. The resin bandage was fixed with medical tape to prevent the rabbit from tearing. The normal group was reared and grasped. After 6 weeks, the resin bandage was removed.

## Acupotomy Interventions

After modeling, the rabbits were fixed gently on the treatment table. The tendon insertion of vastus medialis, vastus lateralis, rectus femoris, biceps femoris, and anserine bursa was selected as treatment points in rabbits. After skin preparation, the sharp of acupotomy needle (Hanzhang brand disposable acupotomy needle, 0.3 × 30mm) was kept parallel to the tendon fiber, and the body of needle was perpendicular to the skin. Pressure was applied for a few moments after moving the needle. Rabbits were treated once every 7 days for 3 weeks. The animals in Normal and KOA groups were fed and grasped routinely (the practice was the same as that of the KOA + Apo group).

After acupotomy interventions for 3 weeks, we began to measure the indicators.

## BOLD MRI

### Imaging Acquisition

BOLD MRI was used for the detection of 4 rabbits in each group. The animals were anesthetized with 3% pentobarbital after treatment, and MRI was performed when they reached deep anesthesia after 20–30 min. Measurements were done with Discrovery750W3.0T (GE Medical Systems, Milwaukee, WI). Images of the knees were acquired in the supine position while exposing animals to alternating normoxia (room air) and carbogen (conventionally 95% $O_2$ and 5% $CO_2$) delivered through a face mask at 7 L/min. In previous study, carbogen was found to have higher inter-rabbit reliability than pure 100% oxygen.[30] Scanning parameters: conventional scanning using OSag fs PD (sagittal fat suppression proton weighted localization image) sequence, FOV, NEX 4, slice thickness 4 mm, interval 0 mm, layer number 17, frequency 320, phase 320. T2*MAP scan (BOLD MRI): TR 44.7 ms, TE 0.9–7.6ms, FOV 10 cm, NEX 2, slice thickness 4 mm, interval 0 mm, frequency 96, number of layers 17, phase 96, 8 images per slice.

### Imaging Analysis

The images were uploaded to GE AW workstation, and the R2* and T2* images were obtained by post-processing software. The range of synovium and surrounding tissue was determined in T2*MAP images, the pixel number of region of interest (region of interest, ROI) was fixed between 85 and 90mm$^2$, and the average value of R2* was calculated.

## Intra-articular Pressure Measurement

Intra-articular pressure was measured in vivo. The three-limb tube was connected to the puncture needle, the 10mL syringe (filled with liquid paraffin), and the pressure transducer, respectively. All devices were placed on the same level. The marker pen was used to locate the depression at the lower margin of the patellar ligament. The needle body was quickly inserted at an angle of 45° to the skin of inside part of the knee joint. The values before and right after the insertion were recorded.

## Specimen Collection

All the animals were then sacrificed by overdose of anesthesia after intra-articular pressure measurement. The synovial fluid of the left hind limb of the rabbit was extracted under anesthesia. The skin was prepared, opened, and separated from the subcutaneous tissue to expose the patellar ligament. The knee joint was 45° bent, and the insertion point was at the middle of the patellar ligament and the lower edge of the femur, with a needle sensation of falling after entry. One

milliliter of saline was injected into the knee cavity. After sufficient bending and stretching of the knee joint, the synovial fluid was collected into lyophilization tubes and the samples were centrifuged at 1000 rpm at 4°C for 15 min to remove cells and joint debris, then stored at −80°C. Afterwards, the synovial tissue of the knee was extracted as well. A longitudinal incision was made along both sides of the patellar ligament, followed by a transverse incision at the upper edge of the patella. Tweezers were used to lift the patellar ligament and the tissues, the joint cavity was opened, and the membrane tissue was exposed. A piece of synovial tissue (0.5cm × 0.5cm × 0.1cm) was cut from the intercondylar fossa of the femur with a surgical blade. The tissue was then fixed in 4% para-formaldehyde and stored lyophilized tubes at −80°C separately.

## Morphological Observations Analysis

HE Staining: Morphological changes in synovial tissue were observed by HE staining. The fixed synovial tissue was dehydrated, embedded in paraffin, and sectioned (thickness: 4μm), stained with hematoxylin and eosin, sealed with neutral gum, and observed under a light microscope.

## ELISA Analysis

The contents of IL-1β and TNF-α in synovial fluid were determined by the ELISA method. The frozen synovial fluid was removed and centrifuged to obtain supernatant after being restored to room temperature. The samples were detected according to ELISA kit procedures (Nanjing Jiancheng Bioengineering Institute, Nanjing, China), and the absorbance values were measured at 450nm with an enzyme labelling instrument (Thermo Fisher Scientific, Waltham, USA). The contents of IL-1β and TNF-α were calculated by comparing them with the standard substance. Three wells were set for each index.

## IHC Analysis

The expression of HIF-1α in synovial tissue was observed by IHC staining. Sections were dewaxed, rehydrated, and performed antigen retrieval. Then, it was treated with 3% hydrogen peroxide for 25 min and blocked with 10% normal goat serum for 30 min. They were then incubated overnight with the primary antibody HIF-1α (1:200, Novus, CO, USA) at 4°C. After rewarming, the slices were washed with PBS for 15 min. The HRP-labeled secondary antibody (HRP labeled goat anti-mouse 1:200, Servicebio, Wuhan, China) was dropped and incubated for 50 min. DAB Immunohistochemistry Color Development Kit was used as a chromogenic agent, hematoxylin was counter-dyed, alcohol gradient dehydration was performed, and neutral gum was dropped to seal the slices. High-quality images were captured in 6 fields per sample and detected by Image-Pro Plus 6.0. The integrated optical density (IOD) of HIF-1α was analyzed for statistical treatment.

## Western Blot Analysis

The protein expression of HIF-1α and VEGF in synovial tissue was detected. After grinding, RIPA Lysis Buffer was added and centrifuged at 12,000 rpm/min at 4°C for 20 min. The supernatant was taken and protein concentration was determined by the BCA method; the protein is denatured at 95°C. Following denaturation, proteins were separated using polyacrylamide gel electrophoresis. Proteins were transferred from the gel onto the PVDF membranes and blocked with 5% nonfat dry milk for 2 h. The PVDF membranes were incubated with the first antibody GAPDH (1:10,000, Abcam, Cambridge, UK), HIF-1α Antibody (1:2000, Novus, CO, USA), and VEGF Antibody (1:2000, Proteintech, Chicago, USA) for overnight at 4°C. The secondary antibody (HRP labeled goat anti-mouse 1:10,000, HRP-labeled goat anti-rabbit 1:10,000, Servicebio, Wuhan, China) was incubated at room temperature for 1h. The membranes were exposed to Electrochemiluminescence (ECL) in a darkroom. Image J software was used to analyze the gray level of the target strip of the scanned image, and the results were calculated by GAPDH.

## Statistical Analysis

All data were analyzed by SPSS 20.0 statistical software, and the experimental data were described as mean ± Standard deviation (SD). One-Way ANOVA followed by LSD was used for comparisons between groups that were normally

distributed and had equal variance. Tamhane's T2 method was used for comparisons between multiple groups that were normally distributed and did not have homogeneous variance. A value of $P < 0.05$ was considered statistically significant. Higher significance levels were established at $P < 0.01$.

# Results

## Acupotomy Reduces Intra-Articular Pressure

To observe the effect of acupotomy on intra-articular pressure in KOA, intra-articular pressure of knee joint was measured by pressure transducer. Compared to Normal group, intra-articular pressure was significantly higher in KOA group ($P < 0.01$). Compared with the KOA group, intra-articular pressure of knee joint in KOA + Apo group was significantly decreased ($P < 0.01$) (Figure 1).

## Acupotomy Improves Synovial Hypoxia of KOA Rabbits

To observe the effect of acupotomy on the oxygen environment of synovium in KOA, we examined the animals by BOLD MRI (Figure 2A). Δ R2* (Δ R2* = R2* (carbogen)-R2* (air)) in KOA group was lower than that in Normal group ($P < 0.05$). While Δ R2* in KOA + Apo group was higher than that in KOA group, and the difference was not statistically significant ($P > 0.05$) (Figure 2B). The result of comparison among the three groups before oxygen inhalation: compared with Normal group, the R2* in KOA group was significantly higher ($P < 0.01$), and the R2* in KOA + Apo group was significantly lower than KOA group ($P < 0.01$) (Figure 2C). The expression of HIF-1α in rabbit synovial tissue was detected by IHC. The HIF-1α content in the knee synovial in KOA group was significantly higher than that in Normal group ($P < 0.01$), while the KOA + Apo group was significantly lower than KOA group ($P < 0.01$) (Figure 3A and B). The results of WB were consistent with those of IHC (Figure 3C and D). The results indicated that acupotomy may help to reduce intra-articular pressure, recover the oxygen environment in the articular cavity, and alleviate synovial hypoxia.

## Acupotomy Improves Synovial Inflammation and Angiogenesis of KOA Rabbits

To observe the effect of acupotomy on synovial inflammation and angiogenesis of KOA, HE staining sections were observed: in Normal group, the synovial lining cell layers were arranged neatly without proliferation, inflammatory cell infiltration or vascular proliferation. In KOA group, the synovial lining cell layers were disorganized and proliferated under hyperplasia, with more inflammatory cell infiltration and vascular hyperplasia visible. The synovial hyperplasia, inflammatory cell infiltration and vascular hyperplasia in the KOA+ Apo group were less severe than those in the KOA group (Figure 4A). Krenn pathological scale was used to score the degree of synovial tissue lesions (Figure 4B). The contents of IL-1β and TNF-α in synovial fluid were detected by ELISA. IL-1β and TNF-α were significantly upregulated in the KOA group compared with the normal group ($P < 0.01$), and these trends were reduced by acupotomy (Figure 4C and D). The protein expression of angiogenesis markers VEGF in synovial tissue was tested by Western Blot (Figure 4E and F). The results showed that compared with the Normal group, the protein expressions of VEGF in synovial tissue of the KOA group were



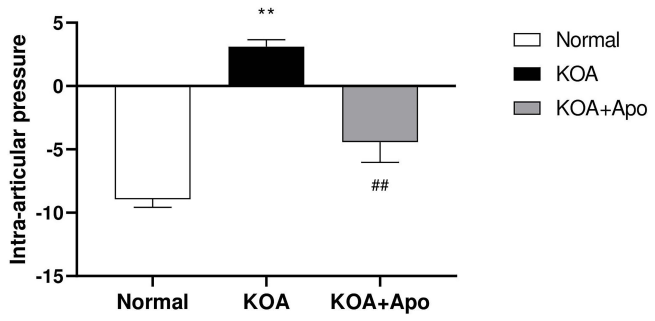


**Figure 1** Effect of acupotomy on intra-articular pressure. The mean resting pressure in each knee. **$P < 0.01$ vs the Normal group. ##$P < 0.01$ vs the KOA group.

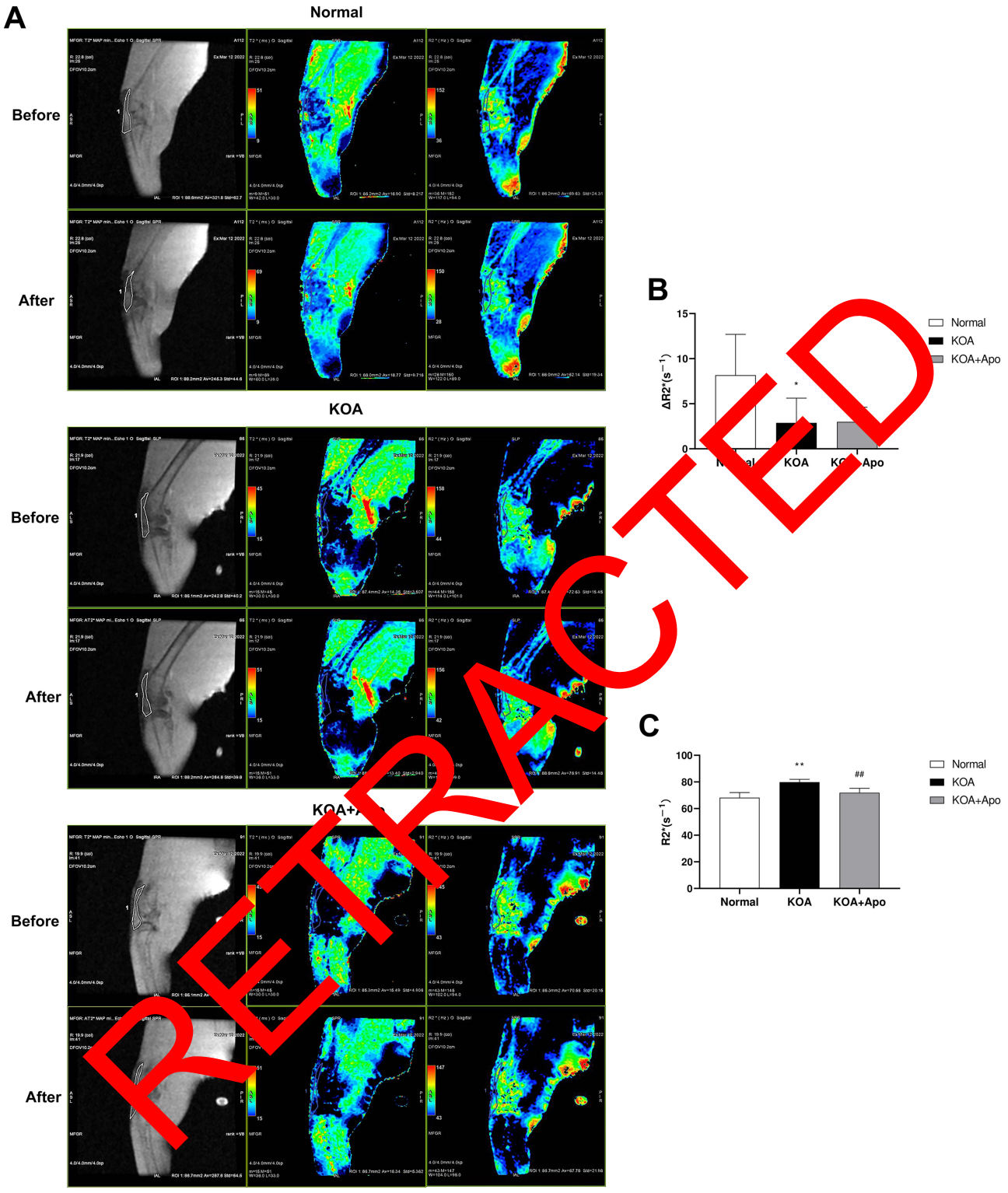




**Figure 2** Acupotomy relieved the state of hypoxia. (**A**) Representative T2* images and pseudo-color T2* images and pseudo-color R2* images in each group. And ROIs used to delineate the synovium to derive reactivity maps of normoxia and hyperoxia (using a mixture of 95% oxygen ($O_2$) and 5% carbogen ($CO_2$) for hyperoxia stimulus). (**B**) Δ R2* changes between groups. *$P$ < 0.05 vs the Normal group. (**C**) R2* changes between groups. **$P$ < 0.01 vs the Normal group. ##$P$ < 0.01 vs the KOA group.

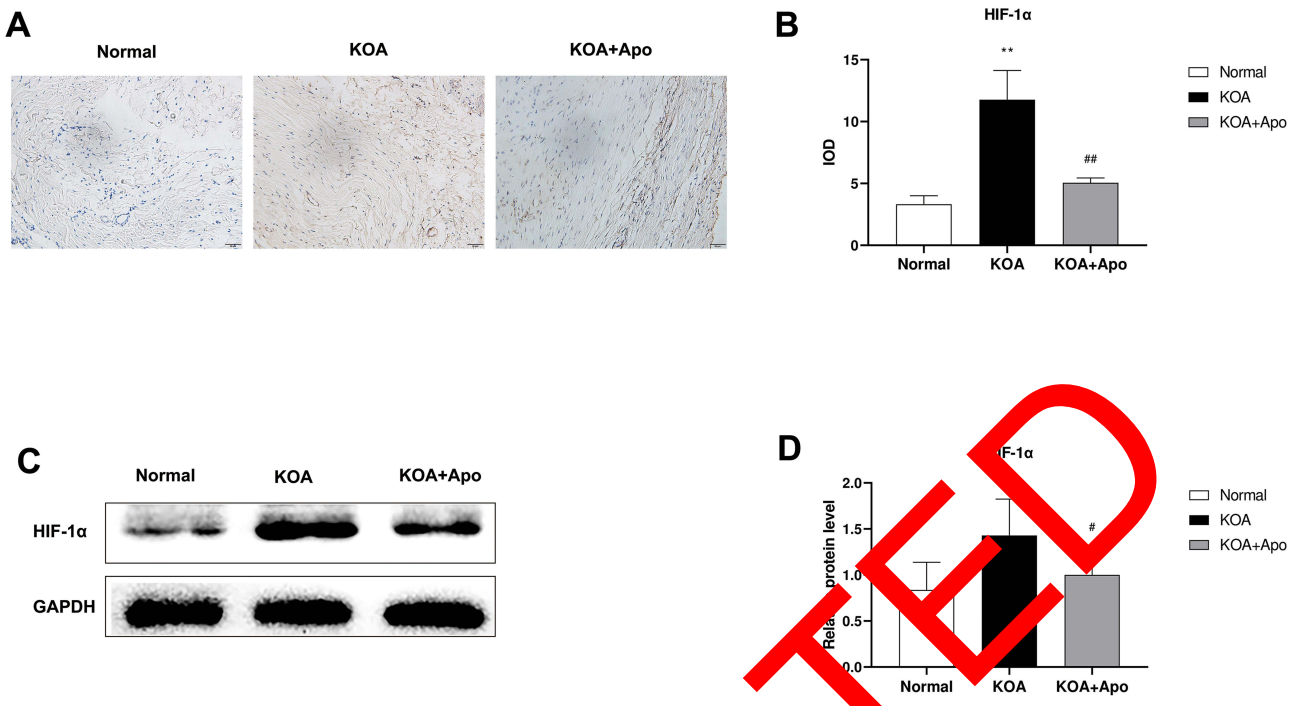


**Figure 3** Acupotomy reduced the expression of HIF-1α in rabbit synovial tissue. (**A**) Representative HIF-1α IHC sections of synovial tissues in each group, 200×. (**B**) Relative IOD value in each group. Semiquantification of IHC sections was evaluated by calculating the positive areas of HIF-1α. Data were analyzed by Image J Pro Plus 6.0. $^{**}P$ <0.01 vs the Normal group. $^{\#\#}P < 0.01$ vs the KOA group. (**C**) Typical protein bands of HIF-1α. (**D**) Protein level comparison of HIF-1α between groups. $^{**}P < 0.01$ vs the Normal group. $^{\#}P < 0.05$ vs the KOA group. Data were analyzed by Image J.



significantly increased ($P < 0.01$); the protein expression of VEGF was decreased ($P < 0.05$). In summary, acupotomy could help to improve synovial inflammation and angiogenesis of KOA rabbits.

## Discussion

This study suggested that acupotomy could alleviate synovial inflammation and slow down the process of angiogenesis in KOA rabbits, which was specifically manifested as the decreased contents of IL-1β and TNF-α in synovial fluid and VEGF in synovial tissue after acupotomy. Knee immobilization increased intra-articular pressure of the animals, decreased partial pressure of oxygen in synovial tissue and resulted in the hypoxia of synovium, allowing an over-accumulation of HIF-1α which triggered many changes that could be reversed by acupotomy. The findings implied that acupotomy may reduce synovial inflammation and angiogenesis in KOA by improving intra-articular hypoxia.

Joint immobilization is one of the commonly used methods for the preparation of KOA experimental models, which could result in muscle atrophy and adhesion of the soft tissues around the joint, and cartilage degeneration, all of which would in return result KOA with the abnormal mechanical stress. The findings of the literature are consistent with our previous studies.[31] Acupotomy could release the muscle and tendon around the knee joint of rabbits to restore the biomechanical characteristics of the joint based on the Jingjin theory of traditional Chinese medicine and the theory of biomechanics and anatomy in modern medicine.[32] This study applied the modified Videman method for KOA modeling, which may be more suitable to explore the related mechanism of acupotomy in the treatment of KOA.

KOA is not merely a passive degenerative disease commonly referred to as "wear -and - tear", but its pathophysiology is more complex. It is a total joint disease caused by the imbalance between the repair and destruction of joint tissues.[33,34] Intra-articular pressure is a direct reflection of the joint stress, and the abnormal joint stress environments may result in a significant increase in intra-articular pressure.[35] The results indicated a significant increase in

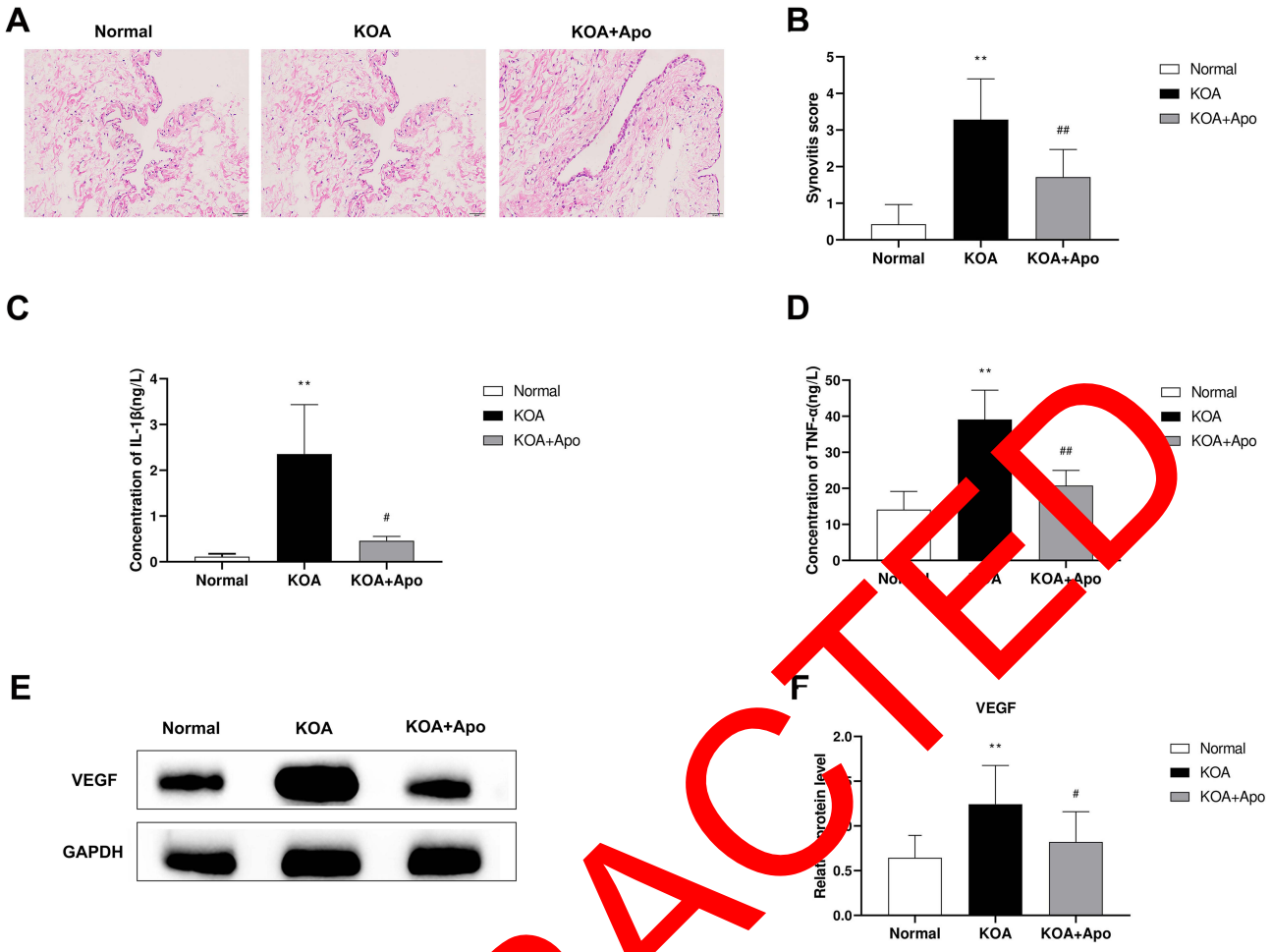


**Figure 4** Acupotomy improved synovial inflammation and angiogenesis. (**A**) Representative synovial tissues of each group stained with HE staining, 200×. (**B**) Krenn pathological scale in each group. $^{**}P < 0.01$ vs the Normal group. $^{\#\#}P < 0.01$ vs the KOA group. (**C** and **D**) The concentration of IL-1β and TNF-α in rabbits in each group. $^{**}P < 0.01$ vs the Normal group. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ vs the KOA group. (**E**) Typical protein bands for each group. (**F**) Protein level comparison of VEGF between groups. $^{**}P < 0.01$ vs the Normal group. $^{\#}P < 0.05$ vs the KOA group. Data were analyzed by Image J.



intraarticular pressure in KOA group and a decrease after acupotomy, which may be related to restored biomechanical characteristics and release of muscle and tendon around the knee joint.

Hypoxia gains increasing attention in current studies of joint problems.[36] We have reasons to believe that the abnormally increased intra-articular pressure is the key to the obstruction of synovial oxygen transmission. The synovium and articular cartilage form a closed environment, and the pressure is transmitted directly to the synovium vasculature. Significantly higher intra-articular pressure exists in the KOA cavity, and due to the tamponade effect, the blood flow velocity of capillaries was reduced, the synovial microcirculation was blocked, and the effective perfusion in the joint cavity was reduced; thus, the repeated and persistent hypoperfusion resulted in joint hypoxia.[21,37] In this study, BOLD MRI and synovial HIF-1α were used to detect hypoxia in the articular cavity of KOA. Previous studies have shown that BOLD MRI is sensitive to microvascular changes in the tissue around the synovium, which may be related to higher concentrations of intravenous DHb in perisynovial tissues with high metabolism and inflammation and in relatively hypoxic joint environments.[17,38] Additionally, due to the increase of intra-articular pressure and the increase of metabolic demand under the influence of inflammation, compensatory perfusion of synovial microvascular system in KOA should carry more DHb. Therefore, we chose BOLD MRI to measure the oxygen partial pressure in synovial microvessels. The quantitative parameter R2* was negatively correlated with oxygen partial pressure. The R2* in KOA group was higher than that in Normal group, indicating that acupotomy might increase the partial pressure of oxygen in the synovium, which was reduced in the KOA group. It is worth noting that we should pay attention to the relationship between IFP and

synovium. These two structures are actually an anatomo-functional unit, and both of them have rich blood flow,[39] so ROIs cannot completely distinguish them. Therefore, we evaluated the oxygen partial pressure of synovium and surrounding tissues. A series of intracellular processes were triggered as well, among which HIF-1α was the most critical transcription factor which mediated the cellular hypoxic response.[40] Under conditions in which $O_2$ supply is limited, HIF-1α subunits accumulate and activate transcription of hypoxia-response elements (HRE)-containing genes.[41] Some common target genes include VEGF and erythro-Poietin.[42] In this study, with the detection of intra-articular pressure and the quantitative analysis of synovial BOLD MRI results and synovial HIF-1α before and after intervention in each group, it was found that acupotomy intervention significantly improved synovial hypoxia, which suggested that acupotomy intervention could reduce intra-articular pressure, improve synovial capillary blood oxygen levels, and reduce the accumulation of HIF-1α.

Angiogenesis and inflammatory activity initiate and perpetuate joint lesions during the development of KOA, causing persistent chronic inflammation, joint damage and pain.[43] Hypoxia may be a crucial persistent factor of synovitis and angiogenesis in KOA. During the pathogenesis of KOA, an overactivation of fibroblast secretes the essential inflammatory mediators IL-1β and TNF-α, which maintain the inflammatory environment of joint.[6,44] A crucial part of the pathogenesis of KOA is played by VEGF, whose expression is increased in synovium macrophages and fibroblast-like synoviocytes.[45,46] VEGF could increase vascular permeability and promote synovial angiogenesis. However, the newly formed blood vessels are immature and cannot keep pace with the proliferation and thickening of synovial tissue.[47] Additionally, VEGF can promote synovial endothelial cell activation to trigger inflammation, demonstrating a connection between angiogenesis and inflammation.[48] The synovial lining cell layers were disorganized and proliferated under hyperplasia, with more inflammatory cell infiltration and vascular hyperplasia visible in KOA group with HE staining. The expression of IL-1β, TNF-α and VEGF was higher in KOA group. Therefore, inhibiting the release of two inflammatory mediators and VEGF is reliable to delay the progression of KOA. HIF-1α was increased in the synovium of KOA rats, and the inhibition of HIF-1α could reduce the expression of inflammation-related factors and VEGF.[49] HIF-1α is involved in synovial inflammation.[50] Hypoxia may occur in early stage of KOA, and inflammatory activation may be one of the consequences of HIF-1α accumulation.[16,51] Synovial inflammation and hyperplasia would increase oxygen consumption and aggravate local hypoxia. The accumulation of IL-1β and TNF-α can lead to the exponential growth of HIF-1α in synovial tissue and promote secretion of VEGF and result in a vicious cycle.[52] As a transcription factor, HIF-1α is associated with the upregulation of VEGF in KOA synovium, which is involved in mediating synovial angiogenesis.[54] In this study, it was suggested that acupotomy may effectively reduce the contents of IL-1β and TNF-α in the synovial fluid and decrease the expression of VEGF in synovium of KOA and a significant improvement in inflammatory and angiogenesis changes in synovial tissue after acupotomy treatment by HE staining was found as well. The changes may be related to the reduction of HIF-1α.

BOLD MRI signal may be regulated by various factors as well, including vascular volume fraction, local hematocrit, blood and tissue oxygenation, vascular structure, vascular maturity, etc.[55] High-oxygen gas mixtures-carbogen (conventionally 95% $O_2$ and 5% $CO_2$) was provided due to the dilate of mature blood vessels of normal tissue after inhaling carbogen gas, and the increase of blood oxygenation will reduce the concentration of DHb.[56] Significant ΔR2* in Normal group was also observed. However, ΔR2* in KOA group was significantly lower than that in Normal group, which would be related to more immature blood vessels in the synovium under hypoxic conditions, and was also confirmed by the changes of HIF-1α and VEGF in this study. Studies have described the changes of ΔR2* in tumor diseases.[57,58] The proliferative and invasive nature of arthritis synovium has frequently been compared to tumor development, both of them exhibit the apparent paradoxical features of hypoxia and angiogenesis.[59] The space of rabbit's knee joints was narrow, thus the periarticular aspect of the knee rather than the synovial microvascular response was evaluated.

This study has some limitations. First, because we did not perform a longitudinal observation but only measured the pertinent study indicators at a single time point, we are unable to explain how oxygen levels in the synovium fluctuate over time. Secondly, the effects of anesthetics on blood volume and blood flow changes were not taken into account for

the evaluation of BOLD MRI values obtained in this study. As a result, the internal effectiveness of our study was also impacted by the failure to evaluate additional sources of measurement errors.

## Conclusion

In summary, this study suggests that acupotomy may help to reduce intra-articular pressure, restore synovial oxygen transport, block HIF-1α accumulation and reduce inflammation-related factor expression and reduce the expression of VEGF, thereby improve the synovitis and angiogenesis in KOA. The results may offer a more advantageous theoretical foundation for the treatment of KOA with acupotomy.

## Abbreviations

Apo, Acupotomy; DHb, Deoxyhemoglobin; ROI, regions of interest; IHC, Immunohistochemical; WB, Western Blot; IOD, integrated optical density.

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## Disclosure

The authors report no conflicts of interest in this work.



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