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ORIGINAL RESEARCH

Therapeutic Efficacy of Oblique Round-Edged Needles in Knee Osteoarthritis: A Mechanistic Study on Muscle and Cartilage Repair

Pengfei Liu[1,*], Yuan Cui[1], Peisong Qin[1,*], Jianye Lv[2], Lingfei An[3], Xiongfei Zhang[3], Ha Si[3], Yelin Wang[3], Leigang Zheng[1,3], Zhifeng Zhang[4]

[1]College of Traditional Chinese Medicine Clinical Medicine, Inner Mongolia Medical University, Hohhot, People's Republic of China; [2]Department of articular surgery, Cangzhou Hospital of Integrated Traditional Chinese and Western Medicine, Cangzhou, People's Republic of China; [3]Department of Orthopedics and Traumatology, Inner Mongolia Hospital of Traditional Chinese Medicine, Hohhot, People's Republic of China; [4]Department of articular surgery, Second Affiliated Hospital of Inner Mongolia Medical University, Hohhot, People's Republic of China

*These authors contributed equally to this work

Correspondence: Leigang Zheng, Department of Orthopedics and Traumatology, Inner Mongolia Hospital of Traditional Chinese Medicine, 11 Jiankang Road, Hohhot, Inner, Mongolia, 010020, People's Republic of China, Email zhengleigang2000@163.com

Purpose: The oblique round-edged needle therapy based on the traditional Chinese medicine theory of "muscle bone balance" is beneficial for KOA. This study observed the behavior of KOA model rats, the structure of rectus femoris muscle and cartilage, and demonstrated that oblique round-edged needle therapy protects the knee joint and alleviates the progression of KOA by relaxing the tendons and muscles around the knee joint.

Animals and Methods: The model used is papain induced KOA in the left hind limb of rats. The rats were divided into a normal group, a model group, an electroacupuncture group, and an oblique round-edged needle group, and each group received 3 weeks of treatment. We evaluated the LequesneMG score and range of motion of the knee joint. Slices of rectus femoris muscle and articular cartilage were stained with hematoxylin-eosin.

Results: Compared with the model group and the electroacupuncture group, the LequesneMG behavioral score and passive range of motion of the knee joint were higher in the oblique round-edged needle group, while the Mankin score of the cartilage was lower.

Conclusion: The oblique round-edged needle improved the LequesneMG score and joint mobility of KOA rats, reducing damage to cartilage structure and delaying the progression of KOA by minimizing rectus femoris muscle injury.

Keywords: articular cartilage, oblique round-edged needle, knee osteoarthritis, rectus femoris

Introduction

Knee osteoarthritis (KOA) is a prevalent chronic degenerative condition, particularly in middle-aged and older populations, with a significant prevalence that adversely impacts patients' quality of life.[1] Knee osteoarthritis (KOA) is primarily defined by the degeneration of articular cartilage, osteomalacia, and inflammatory responses in adjacent tissues, leading to pain, joint stiffness, and dysfunction.[2] Its significant disability is linked to the necessity of knee arthroplasty at the disease's advanced stage, imposing a substantial medical burden on society.[3] Numerous epidemiological studies indicate that roughly 305 million individuals globally are afflicted by this condition, exhibiting a greater incidence in females compared to males, and a notable escalation with advancing age.[4] In China, it is estimated that about 15% of those aged 60 and over are afflicted with the condition.[5]

The traditional treatment methods for knee osteoarthritis include drug therapy, physical rehabilitation, and surgical treatment, but these methods often have limitations or adverse reactions. In recent years, with the deepening of meridian theory and anatomical research, oblique round-edged needles have gradually been applied as a new type of minimally invasive therapy for the treatment of knee osteoarthritis. In traditional Chinese medicine theory, "musculoskeletal

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balance" is an important guarantee for maintaining normal joint function. Therefore, "muscle and bone imbalance" is the key pathogenesis of KOA. Injuries to bones or tendons will inevitably affect the other party, and the extensor muscle group, mainly the quadriceps femoris, is the main pathological tissue in KOA patients. Therefore, we chose the muscle nodes near the rectus femoris tendon and knee joint as treatment points.[6] The oblique round-edged needle is a kind of needle tool between needle knife and acupuncture. It was designed and developed by Jingang Yu, chief physician of Inner Mongolia Hospital of Traditional Chinese Medicine. In 2007, the oblique round-edged needle was granted China's national new design patent (patent number: CN200620047476.1). It has the effect of relaxing muscles, accurately locating the affected area through the principle of "adjusting muscles to treat bones", reducing pain, and improving patient mobility. This study examined the LequesneMG behavioral score and passive range of motion of rat knee joints in a KOA model and investigated the morphological effects on the repair of rectus femoris injury and articular cartilage injury to determine whether treatment has a protective effect on rectus femoris and articular cartilage.

Materials and Methods

Experimental Animals

The SPF grade, 3-month-old, healthy SD rats comprised 40 individuals, evenly divided by sex, with each rat weighing between 200–230 g. The rats were purchased from Beijing Viton Lihua Laboratory Animal Technology Co., Ltd., housed at the Animal Breeding Center of Inner Mongolia Medical University under production license No. SCXK (Beijing) 2021–0011, housed in a single cage, provided free access to drinking water and food, and were irradiated with natural sunlight. This study was approved by the Ethics Committee of Inner Mongolia Medical University (Ethics Approval No. 20240619–03). Meanwhile, we strictly follow the ethical and welfare requirements for the protection of experimental animals (GB/T 35892–2018), and abide by the "3R" principle of "Reduce, Replace, Optimize" to use experimental animals in a scientific, reasonable and humane way.

Main Reagents and Instruments

Dehydrator (Wuhan Junjie Electronics Co., Ltd.), embedding machine (Wuhan Junjie Electronics Co., Ltd.), pathology sectioning machine (Shanghai Leica Instruments Co., Ltd.), freezing table (Wuhan Junjie Electronics Co., Ltd.), tissue spreading machine (Wuhan Junjie Electronics Co., Ltd.), oven (Tianjin Lai Bory Instrument Co., Ltd.), slides (Shitai), imaging system (Nikon, Japan), orthogonal white light photomicrographs (Nikon), scanning and browsing software (3DHISTECH), panoramic slice scanner (3DHISTECH), disposable acupuncture needles (Changchun Aikang Medical Instrument Co., Ltd.), disposable oblique round-edged needles (Jiangxi LaoZongMedicine Medical Instrument Co., Ltd.), joint angle ruler for rehabilitation function evaluation (LNGA LAND GRE), paraformaldehyde (LNGA LAND GRE), and other medical equipment (GRE), paraformaldehyde (Sinopharm Group Chemical Reagent Co., Ltd.), anhydrous ethanol (Sinopharm Group Chemical Reagent Co., Ltd.), xylene (Sinopharm Group Chemical Reagent Co., Ltd.), hematoxylin dye (Beijing Soleil Bao Technology Co., Ltd.), eosin dye (Beijing Soleil Bao Technology Co., Ltd.), hydrochloric acid (Sinopharm Group Chemical Reagent Co., Ltd.), ammonia (Sinopharm Group Chemical Reagent Co., Ltd.), and neutral gum (Beijing Soleil Bao Technology Co., Ltd.) Ltd.), neutral gum (Beijing Solebao Technology Co., Ltd).

Establishment of a KOA Model in Rats and Model Evaluation

Forty SPF-grade SD rats were randomly housed for one week and thereafter divided into 10 normal rats and 30 model rats. The untreated rats served as the normal group, whereas the model rats were referenced from prior experiments: 4% papain solution and L-cysteine solution (0.03 mol/L) were mixed 2:1 for 0.5 h. At the same time, the rats were anesthetized with 3% sodium pentobarbital at 40 mg/kg intraperitoneally and then fixed in the supine position on the operating table. The left knee and its adjacent fur were shaved, thoroughly cleansed, and sterilized with iodophor. The left knee was slightly flexed, and a 1 mL syringe was employed to penetrate the joint cavity obliquely upward from the medial underside of the patella, injecting 0.15 mL of the mixture into the joint cavity. A fresh mixture was prepared for injection on the 1st, 4th, and 7th days of modeling, and the rat model was reared in captivity on the 7th day of the modeling after the injection for a 4-week induction period.

Upon completion, the model group was randomly allocated into three subgroups: the model group, electroacupuncture group, and oblique round-edged needle group, each consisting of 10 rats. A rat was randomly chosen from each of the normal group, model group, electroacupuncture group, and oblique round-edged needle group, and the cartilage of the left knee joint underwent HE staining to examine the cartilage structure and confirm the model's performance.

Intervention and Treatment

Group A (normal group): Standard nourishment over the identical timeframe.

Group B (model group): Standard parenting following successful modeling without intervention.

Group C (electroacupuncture group): After modeling, electroacupuncture intervention was performed at acupoints Yinlingquan (located on the inner side of the hind limb and below the medial condyle of the tibia), Yanglingquan (located on the lower front of the fibular head and about 5 mm on the outer side of the posterior third mile), Neiqiaoyan (located on the inner side of the patella and below the patellar ligament in the hind limb), and Calnose (located on the outer side of the patellar ligament and below the patellar bone in the hind limb). The needle tip was vertically inserted into the muscle layer of about 5 mm, and then connected to an electroacupuncture device to output a sparse wave pattern with a frequency of 2/100 Hz, maintaining a constant current stimulation of 3 mA. The treatment time was 20 minutes per session, with 3 treatments per week for a total of 3 weeks.

Group D (oblique round-edged needle group): After molding, the oblique round-edged needle intervenes, with the blade parallel to the rectus femoris muscle and the needle tip inserted vertically. After entering the muscle layer of about 5 millimeters, clear the tendon points longitudinally three times. After surgery, press the area with a sterile cotton ball for 1 minute to stop bleeding. Treat 3 times a week for a total of 3 weeks.

Text Indices

Behavioral Observations and Lequesne MG Scores

Upon conclusion of the intervention, the Lequesne MG[7] scale was employed to assess the severity of pain, gait, range of motion, and joint swelling in the left knee joint of each rat group, with elevated scores reflecting a more serious lesion.

Assessment of the Range of Motion of the Passive Knee

The passive mobility range of the knee joint was assessed by the same qualified physician in rats from the normal group, model group, electroacupuncture group, needle knife group, and oblique round-edged needle group both post-modeling (pre-treatment) and post-treatment.[8] The passive mobility of the rat's left knee joint was assessed using a medical goniometer, which was utilized as follows: Initially, one end of the protractor was secured parallel to the left femur of the rat, guaranteeing alignment of the protractor's axis with the axis of the knee joint's movement. Subsequently, the left knee joint of each rat was passively extended and flexed, while the protractor was calibrated in accordance with the movement of the tibia. Upon full extension of the knee, the angle was documented as the maximum extension angle; conversely, upon full flexion, the angle was noted as the maximum flexion angle. Passive mobility of the knee is defined as the difference between the maximum flexion angle and the greatest extension angle.

Light Microscopic Observation of HE Staining of the Rectus Femoris Muscle

Before sampling, all rats underwent a 12-hour fast, and the necessary surgical equipment were sterilized at elevated temperatures beforehand. Anesthesia was delivered with an intraperitoneal injection of 3% sodium pentobarbital at a dosage of 40 mg/kg. Anesthesia was delivered via intraperitoneal injection. The skin of the left hind limb up to the ankle joint was prepped and sterilized to guarantee the sterility of the operative environment. The rectus femoris muscle specimen above the left knee patella was swiftly excised using a surgical blade, immersed in a 4% paraformaldehyde solution for tissue fixation, dehydrated, embedded, sectioned, and subjected to HE staining. Following dehydration and sealing, the specimen was prepared for observation, and the rats were euthanized through cervical dislocation at the conclusion of the sampling process.

Light Microscopic Observation of HE Staining Knee Cartilage

All rats underwent a 12-hour fasting period prior to sample, and the necessary surgical tools were sterilized at elevated temperatures beforehand. Anesthesia was delivered with an intraperitoneal injection of 3% sodium pentobarbital at a dosage of 40 mg/kg. Prior to sampling, the left hind limb up to the ankle joint of each rat group was locally prepped and sterilized to guarantee a sterile surgical environment. The left knee joint capsule was incised with a surgical blade, the knee cartilage samples were promptly excised, and then immersed in a 4% paraformaldehyde solution for fixation. The samples were then dehydrated, embedded, sectioned, stained with hematoxylin and eosin (HE), dehydrated again, sealed, and subjected to HE staining for observation. Following sampling, the rats were euthanized using medullary dissection. The severity of the articular cartilage lesions was evaluated by the same experienced physician using the Mankin score.[9]

Statistical Methods

Statistical analysis was conducted using SPSS 27.0 (IBM, Chicago, IL, USA) software, with normally distributed metric data represented by M (P25, P75) and non normally distributed metric data represented by M. Single factor analysis of variance (ANOVA) should be used for multiple group comparisons of metric data with normal distribution and homogeneous variance, followed by pairwise comparisons using Bonferroni test. Use Games Howell test for uneven variance; the Kruskal Wallis test is applied to compare non normal distribution econometric data. The difference is statistically significant with $P < 0.05$. Statistical graphs were generated using GraphPad Prism 9.0.

Results

Effect of Oblique Round-Edged Needle Therapy on Knee Mobility as Assessed by the Lequesne MG Score

As shown in Figure 1A, the Lequesne MG behavioral ratings for the knee joint were markedly lower in the model group, electroacupuncture group, and oblique round-edged needle group compared to the normal group ($P < 0.0001$). The scores of the electroacupuncture group and oblique round-edged needle group were significantly reduced post-treatment compared to the model group ($P < 0.0001$). Compared with the electroacupuncture group, the scores of the oblique round-edged needle group were significantly lower, with significant differences ($P < 0.05$).

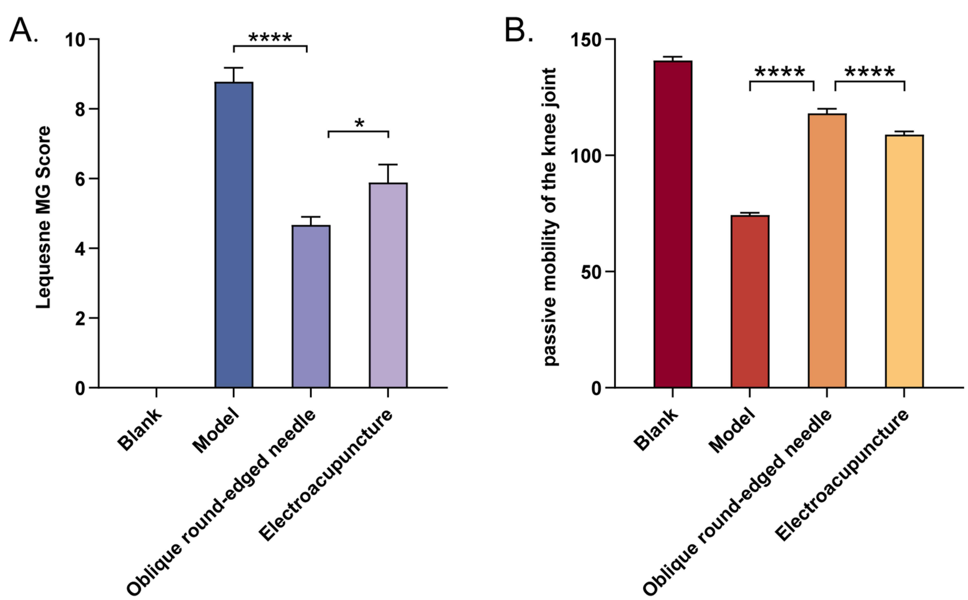

Figure 1 (**A**) Knee mobility was evaluated in each group using the Lequesne MG score, with *P < 0.05 and ****P < 0.0001 indicating significant differences between groups post-treatment; (**B**) Knee mobility was evaluated in each group using the knee passive mobility score, with ****P < 0.0001 indicating significant differences between groups post-treatment.

Effect of Oblique Round-Edged Needle Therapy on Knee Joint Mobility as Assessed by the Passive Range of Knee Mobility

As shown in Figure 1B, the results of knee joint passive mobility revealed that, compared with those of the normal group, the model group, electroacupuncture group, and oblique round-edged needle group were significantly lower after treatment ($P < 0.0001$); compared with the model group, the electroacupuncture group and oblique round-edged needle group were significantly greater after treatment ($P < 0.0001$); compared with the electroacupuncture group, oblique round-edged needle group were significantly greater ($P < 0.0001$).

Effect of Oblique Round-Edged Needle Therapy on the Structure of Articular Cartilage

By observing the HE stained sections (Figure 2A), we found that the cartilage thickness of all normal groups was normal, the surface was regular, the arrangement of chondrocytes was uniform, and the tide line was clearly visible. After treatment, the cartilage layer of the model group showed wear and tear, irregular surface, sparse clusters of chondrocytes,

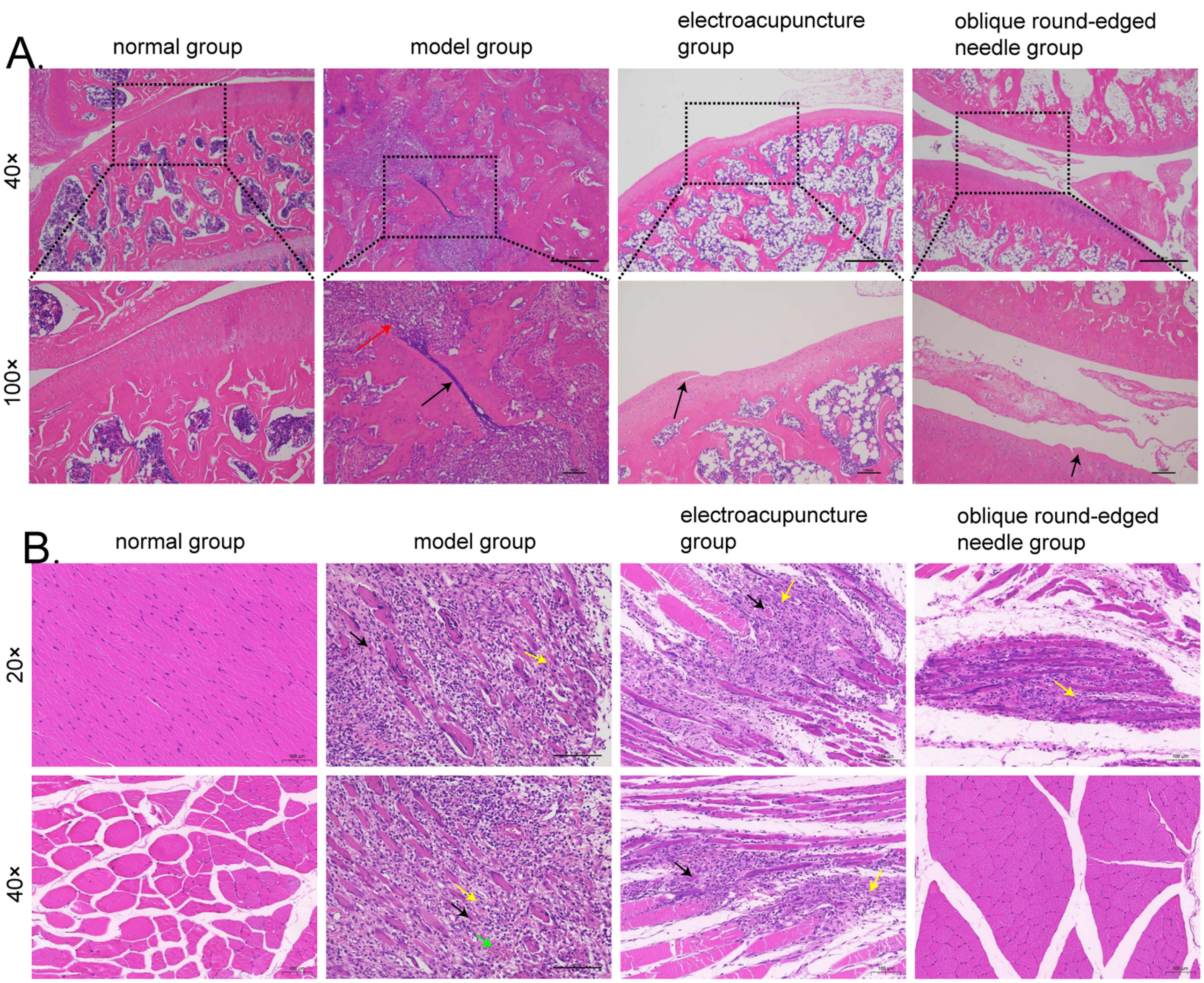

Figure 2 HE staining light microscopy. (**A**) Comparison of chondrocytes in the knee joints of the study groups (×40, ×100). The black arrows represent chondrocytes, the red arrows represent inflammatory cells, and the yellow arrows represent fibrous tissue. In the oblique round-edged needle group, only a slightly uneven surface of articular cartilage was observed, with occasional small fissures, and no significant reduction in chondrocytes was observed; (**B**) Comparison of myocytes in the rectus femoris muscle of the knee joints of the study groups (×20, ×40). The black arrows represent myocytes, the yellow arrows represent fibroblasts, and the green arrows represent hemorrhagic foci. Only a small amount of myocyte necrosis and only a small amount of fibroblast hyperplasia were observed locally in the muscle tissue of the rectus femoris muscle of the rats in the oblique rounded-bladed needle group.

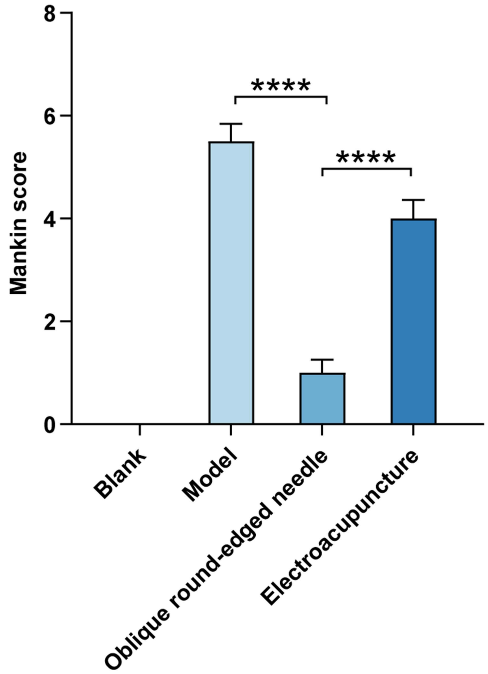

Figure 3 Effect of oblique round-edged needle therapy on the cartilage of the knee joint of rats as assessed by the Mankin score, with ****P < 0.0001 indicating significant differences between groups post-treatment.

almost no visible wrinkles, and severe infiltration of inflammatory cells. Compared with the model group, the electro-acupuncture group retained more cartilage, with a relatively flat surface, less inflammatory cell infiltration, and a basic disappearance of the tide line; Compared with the electroacupuncture group, the oblique round-edge acupuncture group retained more cartilage, had a relatively flat surface, less inflammatory cell infiltration, and still retained the tide line. After treatment, the Mankin scores of the oblique round-edge needle group and the electroacupuncture group were significantly lower than those of the model group ($P < 0.0001$), and the scores of the oblique round-edge needle group were also significantly lower than those of the electroacupuncture group ($P < 0.0001$) (Figure 3).

Effect of Oblique Round-Edged Needle Therapy on the Microstructure of the Rectus Femoris Muscle

By examining the HE stained sections (Figure 2B), we found that the morphology of the normal rectus femoris tissue was normal in both the transverse and longitudinal sections. In the longitudinal section, the muscle cells were elongated cylindrical, and the muscle mass contained many cells arranged parallel to the myogenic fibers along the long axis of the cells. In the transverse section, the muscle fiber bundles were tightly arranged, with most of them being angular. The muscle fibers in the muscle bundle are tightly arranged and mostly angular, with typical light and dark horizontal stripes visible. After treatment, the muscle cells in the model group died, the number of muscle fibers decreased, the number of collagen fibers increased, and the number of fibroblasts increased; Compared with the model group, the oblique round-edged needle treatment group and the electroacupuncture group had less muscle cell necrosis, less collagen fiber increase, and less fibroblast count; Compared with oblique round-edged needle group, the electroacupuncture group had relatively more muscle cell necrosis and more fibroblast proliferation.

Discussion

Osteoarthritis is a multifactorial degenerative disease, and cartilage damage is difficult to recover from.[10] Therefore, delaying disease progression and promptly providing treatment interventions for patients are key to protecting cartilage. The flexion, internal and external rotation, and anterior posterior displacement of the knee joint are related to the coordination between extensor muscle groups including rectus femoris, medial femoris, lateral femoris, and middle femoris, as well as flexor muscle groups such as biceps femoris, semitendinosus, and semimembranosus femoris. Soft tissue damage around the knee joint is directly related to the occurrence of KOA, and muscle dysfunction caused by various pathogenic factors both internally and externally is an independent risk factor for the development of knee osteoarthritis.[11] In recent years, domestic and foreign research has focused on the functional abnormalities of lower limb muscles in patients with KOA, and studies have shown that lower limb extensor muscle weakness, represented by the quadriceps femoris, plays an important role in the occurrence and management of knee osteoarthritis. KOA's oblique round-edged needle therapy usually focuses on the release of ligaments, tendons, and muscles around the patella.[12] According to the theory of "musculoskeletal balance" in traditional Chinese medicine, we start treating bone diseases from the muscles. This study focuses on the tendon points of the rectus femoris muscle and the nodular points of nearby muscles. We found that oblique round-edged needle therapy can delay the progression of osteoarthritis in KOA rats.

In this study, we found that, compared with electroacupuncture treated rats, KOA model rats treated with beveled-blade needling presented better behavioral scores, recovery of joint mobility, and repair of skeletal muscle and articular cartilage. In the HE staining results, the oblique round-edged needle group showed less necrosis of myocytes and proliferation of collagen fibers, and the cartilage surface structure was closer to that of the normal group with clear and intact tidemarks, which is consistent with the concept of "adjusting muscles and treating bones" of the oblique round-edged needle. Possible explanations for this include that the oblique round-edged needle inhibits the release of inflammatory factors and protects the articular cartilage by loosening the adherent fascial tissues and reducing mechanical compression.

In recent years, an increasing number of molecular studies have shown that cyclic mechanical stress promotes chondrocyte proliferation and inhibits apoptosis through the MAPK/ERK signaling pathway.[13] Minimally invasive loosening by beveled-blade needling may mimic this mechanism, thereby delaying cartilage degeneration. We realize that this part of the speculation needs to be supported by further experimental validation, which will be one of the directions of our future research.

This study systematically elucidated the cartilage and muscle protective effects of oblique round edged needles in the KOA model, demonstrating that they can improve the mobility of KOA model rats. Although our research findings have advanced our understanding of the therapeutic potential of oblique round edged needles, there are two key limitations worth considering. Firstly, the current experimental framework does not incorporate functional evaluation through behavioral phenotypes (such as dynamic gait analysis) or advanced imaging modalities (such as μ MRI, ultrasound elastography), which can objectively quantify improvements in joint mechanics and muscle contractility.[14] Secondly, although we have confirmed the protective effects of oblique round blade needle therapy on cartilage and muscle, the molecular pathways through which oblique round blade needle therapy exerts its effects have not been resolved.

Future research should prioritize multimodal functional assessment to link molecular changes with biomechanical outcomes, and combine studies using transcriptomics and other techniques to dissect precise molecular targets of oblique round blade needle therapy. In addition, a system level approach that integrates transcriptomics and proteomics analysis can reveal novel signaling networks regulated by oblique round blade needle therapy. It is crucial to address these knowledge gaps through rigorous multi omics methods and functional validation in order to translate these preclinical findings into targeted therapy strategies for KOA management.

Conclusion

In summary, the oblique needling technique improves histological and behavioral results, indicating its potential application value in muscle cartilage repair. Our results provide evidence that oblique round blade needle therapy can delay the progression of KOA, thereby contributing to the prevention and treatment of KOA from multiple perspectives.

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Disclosure

The authors report no conflicts of interest in this work.

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