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Experimental study of the biomechanics of osteoarthritis of the knee by pulsed electrical stimulation

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Osteoarthritis is one of the most common joint diseases, leading to joint pain, dysfunction, and a reduced quality of life for patients. Therefore, it is particularly important to explore more effective prevention, treatment and management methods to relieve patients' pain and enhance their quality of life. Among physical therapies, pulsed electrical stimulation (PES) is considered to be a promising treatment method due to its high safety and ease-of-use features. PES provides a non-invasive, safe and effective option for patients. However, there are fewer studies on the biomechanical changes of PES in periarticular tissues, and its effects on the biological behavior of chondrocytes remain unknown. This study investigated the effects of PES on the biomechanical properties of osteoarthritic joints and the biological behavior of chondrocytes. The results showed that PES with an intensity of 10 mA and a frequency of 4 Hz increased the cross-sectional area of muscle fibers, prevented muscle atrophy and loss of function, and restored the mechanical properties of muscle tissue. PES also effectively increases the resistivity of knee osteoarthritis cartilage tissue, as well as the elastic modulus of cartilage, which can enhance the biomechanical characteristics of cartilage tissue. PES also promoted the metabolic activity of chondrocytes and increased cartilage matrix synthesis, thereby improving the overall structure and mechanical properties of cartilage tissue. Additionally, cellular experiments showed that 5 consecutive days of 800 mV PES significantly increased the expression level of Piezo1 gene in chondrocytes. At the same time, the expression of type II collagen and transforming growth factor beta increased, while the expression of matrix metallopeptidase 13 decreased. These changes favored the promotion of cartilage matrix synthesis. This has a positive effect on protecting and improving joint health and reducing the impact of osteoarthritis, and is important for understanding the mechanism of action of PES on chondrocytes and the development of related therapeutic strategies.

Knee osteoarthritis, Pulsed electrical stimulation, Biomechanics, Joint muscles, Cartilage

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1. Introduction

Knee osteoarthritis (KOA) is a common chronic disease. It is characterized by gradual deterioration of the knee joint damage and structure, leading to joint pain, dysfunction and reduced quality of life for patients. With the gradual aging of China's population, the prevalence of KOA has been increasing, especially among the elderly and women. A recent meta-analysis found that the overall estimate of the prevalence of symptomatic KOA in China was 14.6% [1]. Data from the United States showed a prevalence of KOA of 7%

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in the adult population aged ≥ 45 years [2]. KOA has become one of the most prevalent joint diseases, with increasing prevalence in different regions based on research data. It is considered a multifactorial chronic disease, with onset and progression involving a combination of genetics, metabolism, joint injury, and inflammation [3]. Knee biomechanics may be a key aspect in understanding KOA pain. During exercise, external forces, muscles and connective tissue exert mechanical loads on joint tissues. These loads interact with cartilage mechanics-biology and are involved in regulating the osteoarthritic process [4]. It has been shown that the loss of knee extensor and flexor strength as well as muscle function is more related to KOA progression and cartilage volume loss [5]. Therefore, repairing the biomechanical properties of the joint is important to slow down the progression of KOA. Currently, treatments for KOA are focused on reducing joint pain and slowing progression. The United Kingdom guidelines of the National Institute for Health and Care Excellence (NICE) guidelines recommend non-pharmacological interventions (i.e., exercise, strength training and weight control) as core treatments aimed at improving the biomechanics of the joints, including exercise or training to increase muscle strength in the knee, weight control, the use of crutches to reduce the absolute forces on the joint, and other direct biomechanical interventions [6].

A relatively safe and non-invasive treatment, pulsed electrical stimulation (PES) for KOA is the use of surface electrodes around the knee joint to generate intermittent, low-intensity electrical impulses to reduce pain, improve joint function, and promote cartilage repair [7]. In the 17th century, Benjamin Franklin used electric shocks to alleviate the symptoms of frozen shoulder [8]. In the 18th century, Italian physician Carlo Galvani discovered that animal muscles and nerves could be contracted by electrical stimulation, laying the foundation for the subsequent development of electrical stimulation therapy [9]. Nowadays, electrical stimulation has become one of the most commonly used treatments in clinical practice and is widely used in the fields of neurological diseases, pain management and muscle rehabilitation. In clinical applications, electrical stimulation can take the form of different types of electrical currents, including direct current, alternating current, pulsed current and pulsed electromagnetic fields. Pulsed currents can be used to achieve different therapeutic effects by adjusting the pulse frequency, width and amplitude [10]. Electrical stimulation has been reported to stimulate chondrocyte proliferation, decrease interleukin-1 expression, upregulate transforming growth factor beta (TGF-β), and promote cartilage matrix synthesis [11]. A clinical study showed positive results of PES in reducing pain and improving function in both a short-term randomized controlled trial and a long-term study [12]. Electrical stimulation reduces spontaneous pain caused by osteoarthritis and achieves the effect of mimicking voluntary movement under normal physiological conditions by stimulating muscle contraction [13]. This is important for improving the function of an injured muscle or muscle group. Despite the great achievements of electrical stimulation in regenerative medicine, its effectiveness in treating KOA remains controversial. Some studies suggest that electrical stimulation may promote bone regeneration and repair, modulate inflammatory responses, and reduce pain [14]. Some studies suggest that electrical stimulation may affect physiological mechanisms at the cellular level, including promoting intracellular metabolic activities, improving cell membrane permeability, and enhancing cellular excitability [15]. In conclusion, the existing studies suggest that electrical stimulation may affect the organism through a variety of pathways, involving joint tissue repair, biological effects at the cellular level, and other dimensions, and the complexity of its mechanism of action increases the difficulty of research and interpretation. Therefore, the effects of PES on the periarticular tissues of osteoarthritic joints as well as the intracellular signaling pathways in chondrocytes need to be further explored to reveal the specific pathways of action in the treatment of osteoarthritis.

The aim of this study was to investigate the effects of PES on the biomechanical properties of joint muscles and articular cartilage in KOA and the potential mechanisms to alleviate cartilage degeneration. *In vivo* experiments were conducted to investigate the effects of PES on the changes in biomechanical properties of articular muscles and cartilage as well as the alleviation of cartilage degradation in KOA. Additionally, the underlying mechanisms of electrical stimulation on the mechanical signaling transformation of chondrocytes were preliminarily explored through *in vitro* experiments. These findings hold significant implications for understanding the therapeutic effects, mechanisms of action, and optimization of treatment protocols involving PES in KOA patients. Furthermore, they furnish a theoretical foundation for its clinical application.

2. Material and methods

2.1 KOA model establishment

To investigate the effect of PES on KOA, we constructed KOA models. This study was approved by the Ethics Committee of Taiyuan University of Science and Technology (Taiyuan, China) and followed the Guidelines for the Protection and Use of Laboratory Animals issued by the Chinese Committee for Animal Science. Adult Sprague-Dawley (SD) rats (8 weeks old, female) were used for the experiment and were randomly divided into a healthy control group (n = 16) and a surgical group (n = 28). The surgical group was randomly subjected to anterior cruciate

ligament amputation on the right knee joint of the rats. After anesthesia, a longitudinal incision of about 3 cm was made on the medial side of the knee joint to expose the joint cavity, and the anterior cruciate ligament was severed, taking care to protect the articular cartilage surface. Positive drawer test indicated that the operation was successful. The joint cavity was rinsed with saline, sutured layer by layer, and aseptically bandaged. Postoperatively, all animals were fed in cages without immobilization of the operated limb and allowed to move freely within the cage. After 12 weeks of observation, four rats from each of the control and surgical groups were selected to verify the success of the KOA model by senna solid green staining. Subsequently, the rats in the surgical group were randomly divided into the KOA group (KOA, n = 12) and the electrical stimulation group (E + KOA, n = 12).

2.2 Intervention method of electrical stimulation in animal model

To investigate the effect of PES on KOA, we applied PES to rat KOA models. After verifying the successful establishment of the KOA model, for the E + KOA group, a digital source meter (Keithley 2450, USA) was used to perform PES treatment in two regions of the right knee joint, the medial and lateral, by means of wires and electrode pads connected to the rat (Fig. 1). The treatment cycle was 3 sessions per week for 4 weeks (12 sessions in total), and the electrical stimulation parameters were as shown in Table 1. For the control and KOA groups, the digital source meter was used in the same way, but the output was switched off during the treatment. Upon completion of the treatment cycle, all animals were euthanized, and the right knee joint of each animal was collected for subsequent studies.

Table 1 Electrical stimulation parameters

Parameter name	Numerical value
Mode	Pulse
Pulse width (μs)	2000
Current intensity (mA)	10
Frequency (Hz)	4
Stimulation time (min)	20
Number of points irradiated	2 points (medial and lateral articular surfaces)
Number of treatments	3 days per week for 4 weeks

2.3 Tensile modulus of elasticity testing of joint muscles

To investigate the effect of PES on the mechanical properties of KOA muscles, we tested the elastic modulus of muscle samples. Firstly, the anatomically collected rectus femoris and soleus muscles were processed to remove the surrounding fat and connective tissue and placed in saline to avoid dehydration and deformation of the tissues. Subsequently, the muscles were wrapped in saline-soaked gauze and stored in a −20 °C refrigerator. Prior to testing, the specimens were thawed at room temperature and the muscle samples were clamped onto the stretching device, ensuring that the clamps held the samples correctly, keeping the muscle ends stretched upwards. The original length of the muscle sample (distance between the two clamps) was recorded using a vernier caliper. Prior to formal testing, the muscle tissue was subjected to 10 repeated loading and unloading cycles to eliminate load creep. The mechanical properties of the muscle samples were tested on a universal testing machine (Instron 3343, USA) at a rate of 40 mm·min^{-1}. The force transducer recorded the tensile force applied to the muscle sample and the elongation during stretching to obtain a load-displacement curve. Calculate the modulus of elasticity of the muscle according to the following equation:

$$E=\frac{\sigma}{\varepsilon}=\frac{\frac{F}{s}}{\frac{\Delta L}{L}}, \tag{1}$$

where s represents the cross-sectional area, and L represents the original length. Sample dimensional parameters were measured three times and averaged. Based on the measured tensile force and displacement data, the stress-strain relationship of the muscle was calculated, and the data points were fitted to obtain its modulus of elasticity.

2.4 HE staining of articular muscles

To study the improvement of muscle function by PES, we

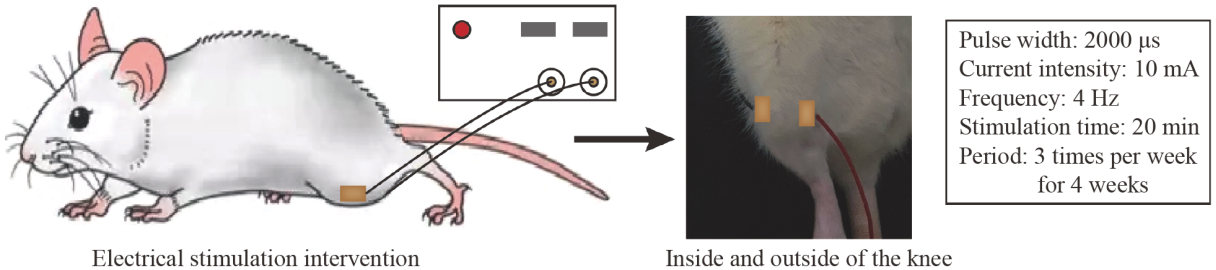

Figure 1 Electrical stimulation for the treatment of KOA in rats.

performed HE staining of the muscles. The fixed-treated muscle tissues were embedded in paraffin, and the paraffin blocks were cut into 5 μm-thick slices using a rotary slicer and placed on clean slides. These sections were then placed in a deparaffinizing agent to remove the paraffin. Then, they were gradually placed in different concentrations of alcohol for rehydration. The sections were then stained in eosin stain for 2 min and subsequently placed in different concentrations of alcohol and hyaluronic acid. Finally, the sections were fixed and sealed. Microscopic images of HE-stained sections were acquired using a digital pathology slide scanner (Pannoramic MIDI, 3DHISTECH, Hungary) at 20× magnification. The number and average cross-sectional area of muscle fibers in the fixed field of view (500 μm × 400 μm) were analyzed using Image J software.

2.5 Electrical impedance test of articular cartilage

To investigate the effect of PES on articular cartilage, we tested the electrical impedance of articular cartilage. In this study, the two-electrode method was used to measure the electrical impedance of cartilage. The impedance testing system consisted of an electrochemical workstation and a computer for data acquisition. A square area of approximately 3 mm × 3 mm in the center of the medial tibia of rats was selected for sample preparation. The remaining portion of the articular cartilage specimen was sanded using sandpaper until it was completely removed. Subsequently, the prepared samples were placed in saline for cold storage. Prior to testing, the specimens were thawed at room temperature. The electrical impedance of each group of cartilage was measured using the two-electrode method: two square copper electrodes with 3 mm sides were placed on the upper and lower surfaces of the specimens so that they coincided perfectly in the vertical direction to ensure that the current flow through the specimens followed a uniform path. Electrical impedance is usually expressed in complex numbers:

$$Z = |Z|\,\mathrm{e}^{j\theta} = |Z|\cos\theta + j|Z|\sin\theta = R + jX, \tag{2}$$

where the magnitude of the electrical impedance $|Z|$ denotes the ratio of the voltage magnitude to the current magnitude, θ is the phase difference between the voltage and the current, j is the imaginary unit, and the real part of the electrical impedance Z is the resistance R, and the imaginary part is the reactance X. The magnitude of the electrical impedance $|Z|$ was measured as the passive electrical properties of the articular cartilage in this study. Each sample retained the full layer of cartilage, so the samples were not exactly the same thickness. In order to make the data more convincing, the electrical impedance of different specimens was normalized according to thickness in this study. The resistivity of the cartilage samples is given by the following equation:

$$\rho = \frac{|Z|B}{L} = \frac{|Z|B}{h}, \tag{3}$$

where ρ is the resistivity of the sample, B is the effective polar plate area of the copper electrode, and L is the distance between the two polar plates, expressed through the thickness (h) of the sample.

2.6 Articular cartilage compression test

To investigate the effect of PES on articular cartilage, we tested the elastic modulus of articular cartilage. Cartilage samples were prepared by removing cylindrical samples using a perforator and sandpapering them from the subchondral bone upwards using sandpaper. The diameter and thickness of each cartilage sample were repeatedly measured and recorded before the start of the experiment, and the average of the three measurements was taken as the final size parameter. In order to eliminate the effect of specimen thickness, the thickness of each set of cartilage specimens was maintained at approximately 0.27 mm and the diameter at approximately 2 mm. The specimens were placed on a universal testing machine (INSTRON 3343) and set to a compression speed of 0.054 $\mathrm{mm\cdot s^{-1}}$ with a compression displacement of 20% of the cartilage thickness. Based on the load-displacement curve, the stress-strain curve was plotted and fitted to the data points to calculate the modulus of elasticity.

2.7 Cartilage staining with Safranin O-Fast Green staining

In order to study the effect of PES on cartilage tissue, we stained the cartilage with Safranin O-Fast Green staining. Samples were fixed in 4% paraformaldehyde for 48 h and decalcified in 10% ethylenediaminetetraacetic acid (EDTA, pH = 7.4), followed by dehydration and paraffin embedding. Consecutive 5 μm thick sections were cut from the central weight-bearing region of the cartilage, and the sections were treated in xylene to remove paraffin and then hydrated through an ethanol series. The sections were then placed in saffron solution for about 5-10 min, followed by solid green solution for about 5 min. The samples were again dehydrated, clarified in xylene and finally sealed. Microscopic images of the sections were collected using a digital pathology slide scanner with 20 times magnification. The histological evaluation of cartilage sections was performed using the Mankin scoring system, analyzing four different parameters with a total score of up to 13: cartilage structure (0-6 points), chondrocyte density (0-3 points), matrix staining (0-4 points) and tidemark integrity (0-1 point).

2.8 Immunohistochemical staining of articular cartilage

To study the effects of PES on cartilage tissue, we performed immunohistochemical staining of cartilage. Paraffin sections were cut as previously described, then routinely dewaxed and hydrated. Sections were incubated with rabbit anti-type II collagen antibody (Proteintech, USA, Cat # 28459-1-AP) and anti-matrix metallopeptidase 13 (anti-MMP-13) antibody (Proteintech, USA, Cat # 18165-1-AP) at 4 °C overnight. Unbound primary antibody was thoroughly washed away using buffer and the sections were incubated with polyserositis anti-mouse/rabbit IgG secondary antibody (Proteintech, USA, Cat # PR30011) for 1 h at room temperature with shaking. Staining was performed using a DAB kit. Microscopic images of immunohistochemical sections were collected using a digital pathology slide scanner (Pannoramic MIDI, 3DHISTECH, Hungary) at 20× magnification.

2.9 Primary chondrocyte extraction and culture

To study the effects of PES on chondrocytes, we extracted chondrocytes. Newborn SD suckling mice of 3-5 d were selected, and the newborn mice were killed by anesthesia. The skin and soft tissues on the hind legs were removed with scissors and pliers to dislocate the femur, and the soft tissues were discarded. The femoral head, femoral condyle and tibial plateau were separated with a scalpel and placed in Dulbecco's Modified Eagle's Medium (DMEM) containing penicillin-streptomycin. A mixture of collagenase D and DMEM was prepared at a ratio of 3 $mg \cdot mL^{-1}$. Cartilage pieces were digested in 10 mL of the mixture and incubated for 45 min. Cartilage fragments were extracted and re-digested for 45 min. Cartilage fragments were extracted and added to 10 mL of the digestion solution (diluted 6×) and incubated for 12 h. The collagenase solution with residual cartilage was extracted and the cells were blown up to obtain the isolated suspension. The suspension was filtered using a 48 μm sterile cell filter and centrifuged. The cells were blown and cultured in DMEM to obtain 2nd or 3rd generation chondrocytes for subsequent experiments.

2.10 Chondrocyte electrical stimulation method

To study the effects of PES on chondrocytes, we applied PES to chondrocytes. The PES device used was designed and fabricated in-house for this experiment. The device consists of an electrical stimulation cover, a programmable signal generator (Juntek PSG9080, China) and two wires. The electrical stimulation cover consists of a 24-well plate cover and 48 electrodes. The 24-well plate cover was drilled and inserted into the electrode pads, and the copper cores of the wires were exposed and attached to the electrode pads. A conductive gel is used to bond the electrode pads, copper wires, and 24-well plate together, with one end of the wire longer than the edge of the plate cover. In use, the desired electrical stimulation parameters can be applied to the cells by connecting the clip on the front of the wire to the end of the wire on the electrical stimulation cover (Fig. 2). In this experiment, a pulsed square wave with a frequency of 2 Hz and a stimulation time of 20 min was used, which was divided into six groups: control and electrical stimulation-treated groups (with output voltages of 50, 100, 200, 400, and 800 mV, respectively). The chondrocyte suspension was first inoculated with 2×10^4 cells/well in a 24-well culture plate, with a total volume of 1 mL per well. The chondrocytes were observed to be completely adherent to the wall, following which they were treated according to the established parameters of the electrical stimulation for 1, 3 or 5 d.

2.11 Detection of chondrocyte proliferation activity by CCK-8 method

To study the effect of PES on chondrocyte activity, we used a CCK-8 assay kit. After 24 h of electrical stimulation, the

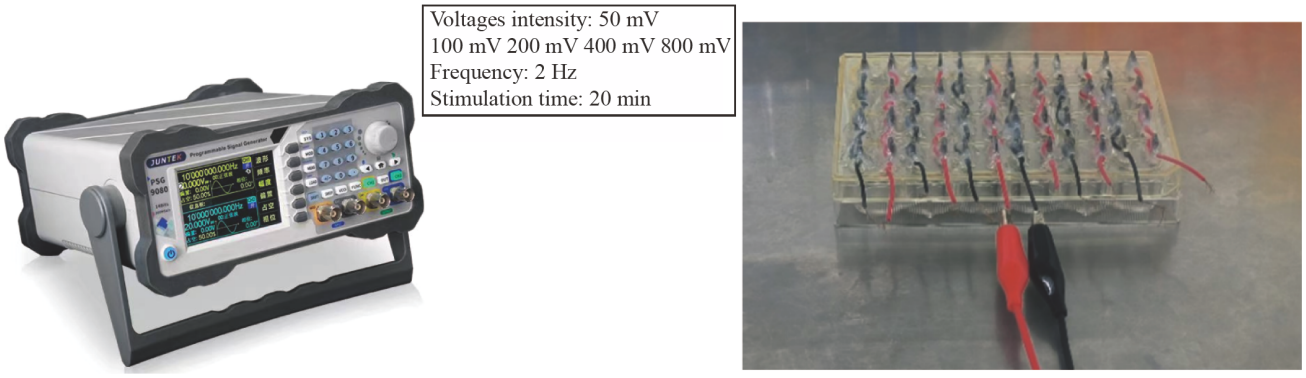

Figure 2 24-well plate electrical stimulation device.

optical density value of each group was measured by CCK-8 method. The specific methods were as follows: prepare the mixture according to the ratio of incomplete culture medium: CCK-8 = 9:1; aspirate the culture medium from the 24-well plate and wash it; add 200 μL of the mixture slowly along the side wall of the wells, and place it in the incubator at constant temperature for 2 h, avoiding light throughout the whole process. The absorbance of the liquid in the 96-well plate was measured at 450 nm using an enzyme counter.

2.12 Total RNA extraction and qRT-PCR of chondrocytes

To investigate the effect of PES on gene expression in chondrocytes, we extracted mRNA from the cells and performed qRT-PCR reactions. Total RNA was extracted according to the instructions of Total RNA Small Volume Extraction Kit (Dual Column). Remove the petri dish, aspirate the culture medium, wash twice with PBS, add 400 μL of lysate to each well and blow. Transfer the mixed liquid into a set of HiPure RNA Column, centrifuge (12000 g, 30-60 s), add 500 μL of Buffer RW1, centrifuge (12000 g, 30-60 s); add 500 μL of Buffer RW2, followed by centrifugation (12000 g, 30-60 s); add 30 μL of RNase Free Water to the center of the column membrane and centrifugation (12000 g, 1 min). Detect the absorbance of the samples at the wavelengths of 260 and 280 nm. The reverse transcription system was set up according to the reverse transcription reagent instructions for reverse transcription, and the qRT-PCR reaction was performed by the quantitative PCR instrument. The primer sequences were obtained from NCBI and synthesized by Shanghai Shenggong Biotechnology Company, and the primer sequences are shown in Table 2.

2.13 Statistical analyses

To assess the statistical significance of the experimental data, statistical analyses were performed using Prism 8.0.2 (GraphPad, CA, USA). Measurements were expressed as mean ± standard deviation. One-way analysis of variance was used and Tukey's multiple comparison test was used to compare significant differences between groups. $P < 0.05$ was considered statistically significant.

3. Results

3.1 Construction of rat KOA model

We stained the histological sections of rat knee joints with senna solid green to observe the structural morphology of knee cartilage. The results showed that the stained cartilage in the control group had normal structure, flat surface, normal number of chondrocytes, and neat arrangement. The cartilage in the KOA group underwent obvious degeneration, with chondrocyte hyperplasia and reduced matrix staining (Fig. 3(a)). Mankin score was significantly higher in the KOA group compared to the control group (Fig. 3(b)). This indicated that we successfully constructed a rat KOA model.

3.2 Effects of PES on muscle biomechanical properties

As shown in Fig. 4(a) and (b), the typical stress-strain curves for each muscle showed a nonlinear exponential relationship, and a straight line (dashed box) was chosen to fit the muscle elastic modulus. As shown in Fig. 4(c), the elastic modulus of rectus femoris muscle of KOA group was higher than that of control group, with no significant difference (0.057 ± 0.018 MPa vs. 0.034 ± 0.012 MPa; $P > 0.05$). In comparison to the KOA group, the elastic modulus of rectus femoris muscle in the E + KOA group exhibited a decreasing trend, although not significantly different (0.047 ± 0.019 MPa vs. 0.057 ± 0.018 MPa, $P > 0.05$). As shown in Fig. 4(d), the elastic modulus of the soleus muscle was significantly higher in the KOA group (0.537 ± 0.094 MPa vs. 0.188 ± 0.127 MPa; $P < 0.01$); while the elastic modulus of the soleus muscle in the E + KOA group was significantly lower in the E + KOA group compared with the KOA group (0.295 ± 0.116 MPa vs. 0.537 ± 0.094 MPa; $P < 0.05$). These results suggest that PES significantly reduced the modulus of elasticity of the soleus muscle and helped to restore the biomechanical properties of the muscle tissue.

3.3 Effects of PES on muscle function

The rectus femoris and soleus muscles play a supportive and protective role, relieve joint loads and reduce cartilage damage. In this study, the rectus femoris muscle (Fig. 5) and the soleus muscle (Fig. 6) were collected for HE staining, and the number of muscle fibers and the cross-sectional area

Table 2 Primer sequences

Gene	Upstream primer (5′-3′)	Downstream primers (5′-3′)
GAPDH	TGCACCACCAACTGCTTAG	GGATGCAGGGATGATGTTC
Piezo1	AGCCATCGATACCAAGGCTC	CTGTACACGGTGCTGACTGT
COL-II	ACAATGTCAGGGCCAGGATG	CAGGACGAGGGCTTCCATAC
TGF-β	GACTCTCCACCTGCAAGACC	GGACTGGCGAGCCTTAGTTT
Aggrecan	GGGACCTGTGTGAGATCGAC	GGTCGGGAAAGTGGCGATAA
MMP-13	GGCCCTGAATGGGTATGACA	TGTCCTCAAAGTGAACCGCA

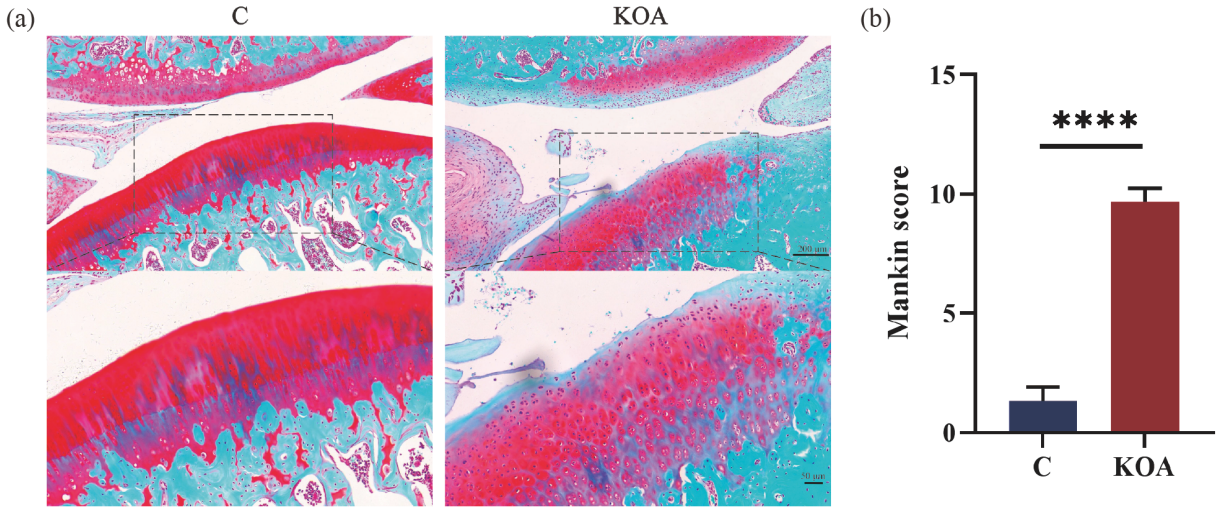

Figure 3 Cartilage histology: (a) representative diagram of senna solid green staining; (b) comparison of Mankin scores for cartilage.

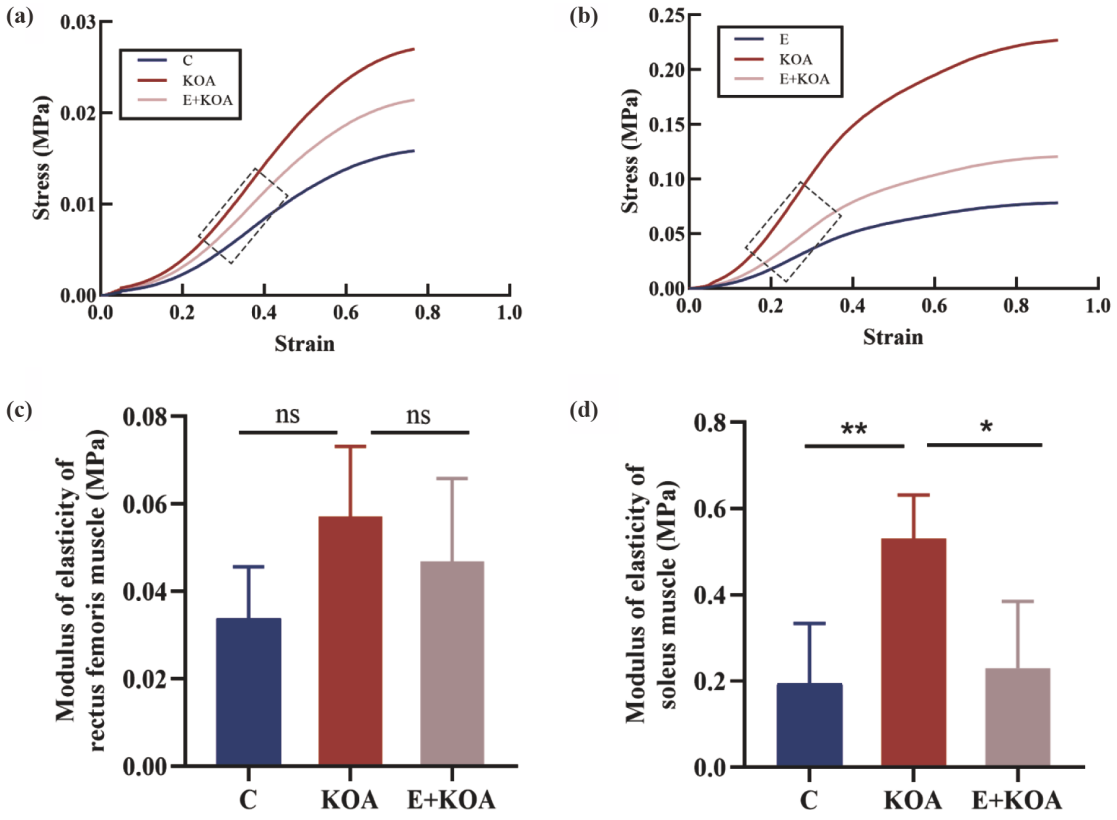

Figure 4 Muscle biomechanical properties: (a) stress-strain curve of rectus femoris muscle; (b) stress-strain curve of soleus muscle; (c) elastic modulus of rectus femoris muscle; (d) elastic modulus of soleus muscle.

of the rectus femoris and soleus muscles were statistically analyzed, respectively. As shown in Fig. 5(a) and (b), the number of muscle fibers of rectus femoris in the KOA group was significantly higher (75.5 ± 8.4 vs. 44.8 ± 12.4; $P < 0.01$) and the mean cross-sectional area was significantly lower (1.34 ± 0.16 vs. 2.8 ± 0.85; $P < 0.01$) than that in the control group; the number of muscle fibers of rectus femoris in the E + KOA group (71.5 ± 9.3 vs. 75.5 ± 8.4; $P > 0.05$) and mean cross-sectional area (1.52 ± 0.13 vs. 1.34 ± 0.16; $P > 0.05$) were not significantly different in the E + KOA group. HE staining of flounder muscle showed that the number of muscle fibers was significantly higher in the KOA group (87.67 ± 4.16 vs. 44.33 ± 3.05; $P = 0.0001$) and the mean cross-sectional area was significantly lower in the KOA group compared to the control group (1.20 ± 0.10 vs. 2.65 ± 0.26; $P < 0.001$); and the number of fibers was sig-

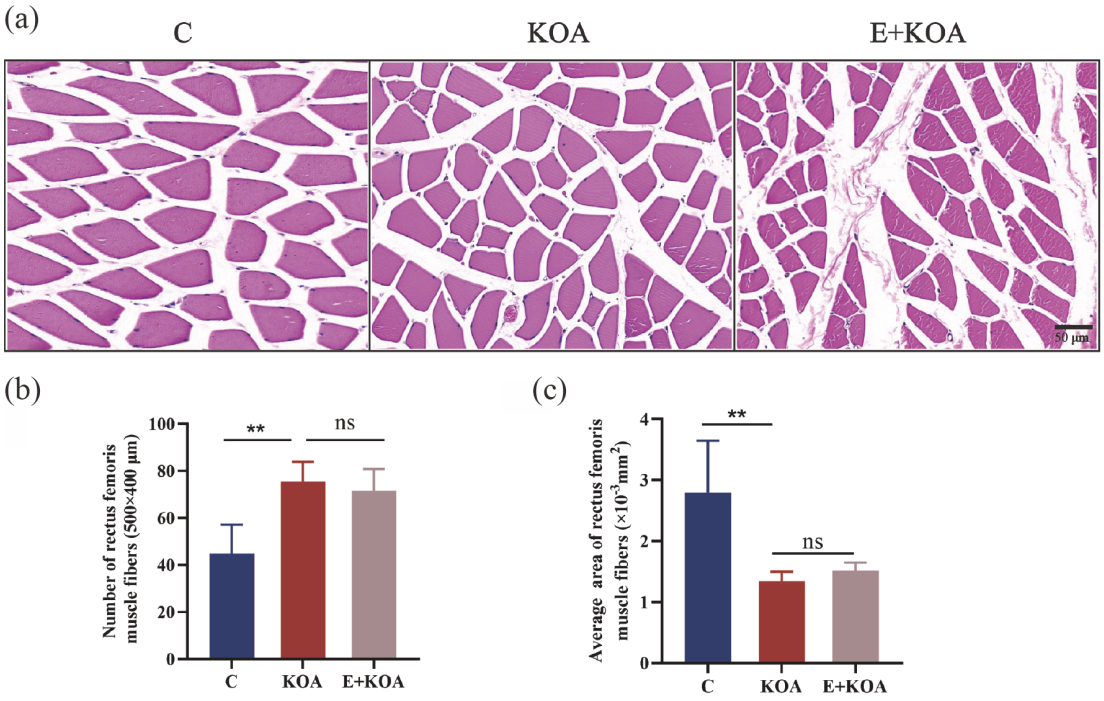

Figure 5 Rectus femoris muscle HE staining. (a) HE staining; (b) number of muscle fibers; (c) average cross-sectional area of muscle fibers.

nificantly lower in the flounder group (58.3 ± 0.4; $P > 0.05$) and the KOA group (58.3 ± 0.16; $P > 0.05$) compared to the KOA group. Compared with the KOA group, the number of flounder muscle fibers in the E + KOA group was significantly lower (58.3 ± 7.6 vs. 87.67 ± 4.16; $P < 0.01$), and the mean cross-sectional area was significantly higher (1.92 ± 0.23 vs. 1.20 ± 0.10; $P < 0.05$), as shown in Fig. 6(a) and (b).

3.4 Effects of PES on the electrical impedance characteristics of articular cartilage

In this study, the effect of PES on the electrical characteristics of cartilage was investigated by measuring the electrical impedance of each group of cartilage using the two-electrode method. As shown in Fig. 7(a), the resistivity of

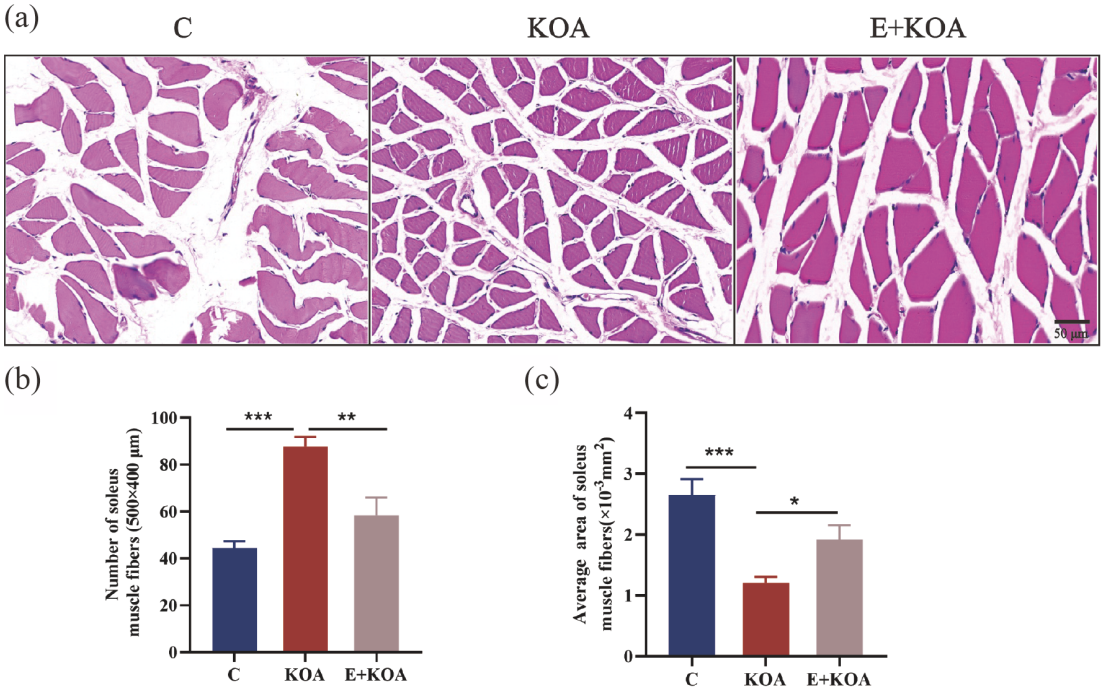

Figure 6 Soleus muscle HE staining. (a) HE staining; (b) number of soleus muscle fibers; (c) average cross-sectional area of soleus muscle fibers.

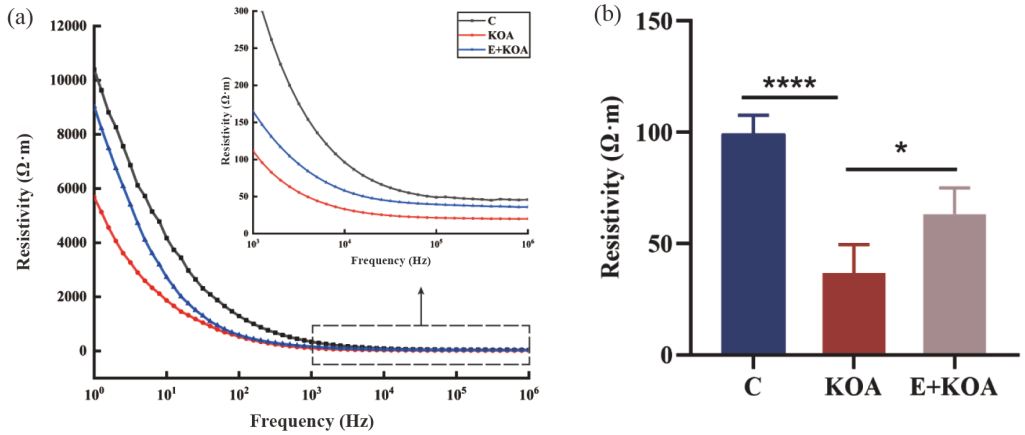

Figure 7 Electrical impedance characteristics of cartilage: (a) electrical impedance graph; (b) resistivity of articular cartilage at 10^4 Hz.

cartilage samples in each group showed a decreasing trend with increasing frequency. Compared with the control group, the resistivity of the KOA group decreased significantly. The E + KOA group showed a significant increase in cartilage resistivity compared to the KOA group. Figure 7(b) analyses the resistivity of cartilage samples at 10^4 Hz. The cartilage resistivity was significantly lower in the KOA group compared to the control group (36.80 ± 12.80 vs. 99.31 ± 8.44 Ω·m; $P < 0.0001$). The cartilage resistivity was significantly higher in the E + KOA group compared with the KOA group (63.17 ± 11.94 vs. 36.80 ± 12.80 Ω·m; $P < 0.05$). These results suggest that PES effectively increased the resistivity of KOA cartilage tissue.

3.5 Effects of PES on biomechanical properties of articular cartilage

As shown in Fig. 8(a), the typical stress-strain curves of each cartilage showed a non-linear exponential relationship, and the straight line (dashed box) was selected to fit the elastic modulus of cartilage. Figure 8(b) demonstrates the cartilage elastic modulus for each group, which was significantly lower in the KOA group compared to the control group (4.33 ± 0.87 vs. 7.28 ± 0.50 MPa; $P < 0.001$). Furthermore, the cartilage elastic modulus was significantly higher in the E + KOA group compared to the KOA group (5.99 ± 0.52 vs. 4.33 ± 0.87 MPa; $P < 0.05$). The results suggest that PES is effective in increasing the elastic modulus of KOA cartilage and enhancing the biomechanical properties of cartilage tissue.

3.6 Effects of PES on the morphology of articular cartilage

We performed senna-solid green staining of histological sections to observe the morphological structure of cartilage. As shown in Fig. 9(a), the cartilage structure of the control group appeared intact, with neatly arranged chondrocytes and uniformly stained matrix. In the KOA group, the cartilage structure was obviously degenerated, the surface of articular cartilage was rough, the intensity of matrix staining was weakened, obvious clefts were seen, and chondrocytes were lost. The cartilage surface of the E + KOA group was

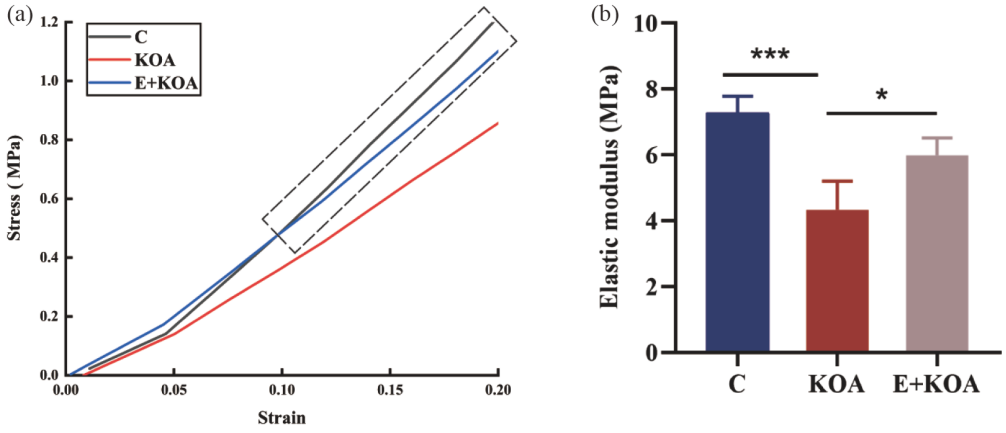

Figure 8 Biomechanical properties of cartilage: (a) stress-strain curve of cartilage; (b) elastic modulus of articular cartilage.

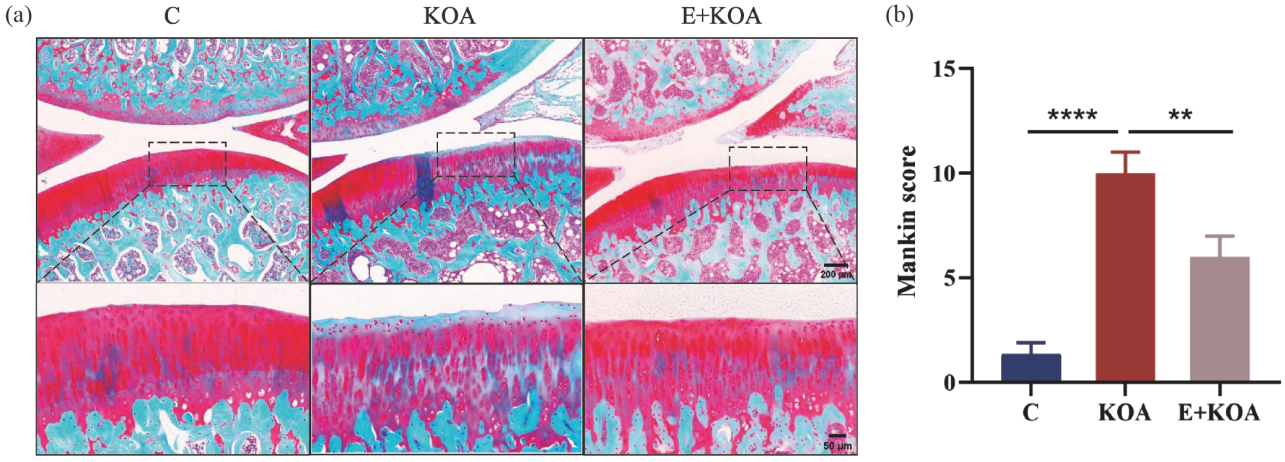

Figure 9 Cartilage histology: (a) representative diagram of senna solid green staining; (b) comparison of Mankin scores for cartilage.

relatively smooth and structurally intact, the number of cells was slightly reduced, and the staining of matrix was uniform, which was obviously improved compared with that of the KOA group. As shown in Fig. 9(b), the Mankin scoring system was used for histological evaluation of cartilage sections. The results showed that the Mankin score was significantly higher in the KOA group compared with the control group (10.00 ± 1.00 vs. 1.33 ± 0.58; $P < 0.001$). The Mankin score was significantly lower in the E + KOA group compared to the KOA group (6.00 ± 1.00 vs. 10.00 ± 1.00; $P < 0.01$). These results suggest that PES can decelerate the process of cartilage structure destruction in KOA and exert a protective effect on cartilage.

3.7 Effects of PES on the expression of type II collagen and MMP-13 in cartilage tissue

In order to further examine the effect of PES on KOA, immunohistochemical staining was used to analyze the expression of type II collagen and MMP-13 *in vivo*. As shown in Fig. 10(a) and (c), the percentage of type II collagen positive area was significantly decreased in the KOA group (1.12 ± 0.13 vs. 2.12 ± 0.18; $P < 0.0001$), while the positive expression of the extracellular matrix disintegration factor, MMP-13 in Fig. 10(b) and (d), was significantly increased (1.20 ± 0.14 vs. 0.25 ± 0.09; $P < 0.0001$), suggesting a decrease in the cartilage matrix of the KOA group. In contrast, type II collagen positive expression was significantly increased in the E + KOA group (1.56 ± 0.18 vs. 1.12 ± 0.13; $P < 0.05$), and MMP-13 expression was significantly decreased in the E + KOA group (0.63 ± 0.20 vs. 1.20 ± 0.14; $P < 0.01$) compared with the KOA group. The above results indicated that PES effectively alleviated the degradation of KOA cartilage matrix by promoting the synthesis of type II collagen and reducing the expression of MMP-13 in cartilage tissue.

3.8 Effects of PES on chondrocyte viability

The chondrocytes were treated with PES at 100, 200, 400 and 800 mV output power for 1, 3 and 5 d, respectively, and the cell viability was measured by CCK-8 24 h after the treatment. As shown in Fig. 11, the viability of chondrocytes was significantly increased by 1, 3 or 5 d of treatment with electrical stimulation ($P < 0.05$). As shown in Fig. 11(a, b), the most significant increase in chondrocyte viability was achieved by applying 800 mV PES ($P < 0.0001$). The results indicated that the electrical stimulation treatment significantly promoted the proliferative viability of chondrocytes.

3.9 Effects of PES on the expression levels of marker genes in chondrocytes

Since the most significant increase in chondrocyte viability was caused by the application of 800 mV PES, we analyzed the effects of this parameter on the mRNA expression of Piezo1, type II collagen, MMP-13 and TGF-β in chondrocytes by qRT-PCR. As shown in Fig. 12, compared with the control group, the expression of Piezo1 and type II collagen in chondrocytes was not significantly different ($P > 0.05$), while the expression of MMP-13 and TGF-β was significantly decreased ($P < 0.05$) by 3 d of pulsed stimulation at 800 mV. The expression of Piezo1, type II collagen and TGF-β in chondrocytes was significantly increased by 5 d of pulsed stimulation at 800 mV. The expression of Piezo1, type II collagen and TGF-β in chondrocytes was significantly increased ($P < 0.05$), while the expression of MMP-13 was significantly decreased ($P < 0.05$). This suggests that 5 consecutive days of PES at 800 mV significantly increased the mRNA expression of Piezo1 in chondrocytes, promoted the expression of type II collagen and TGF-β, and decreased the expression of MMP-13, thus inhibiting the

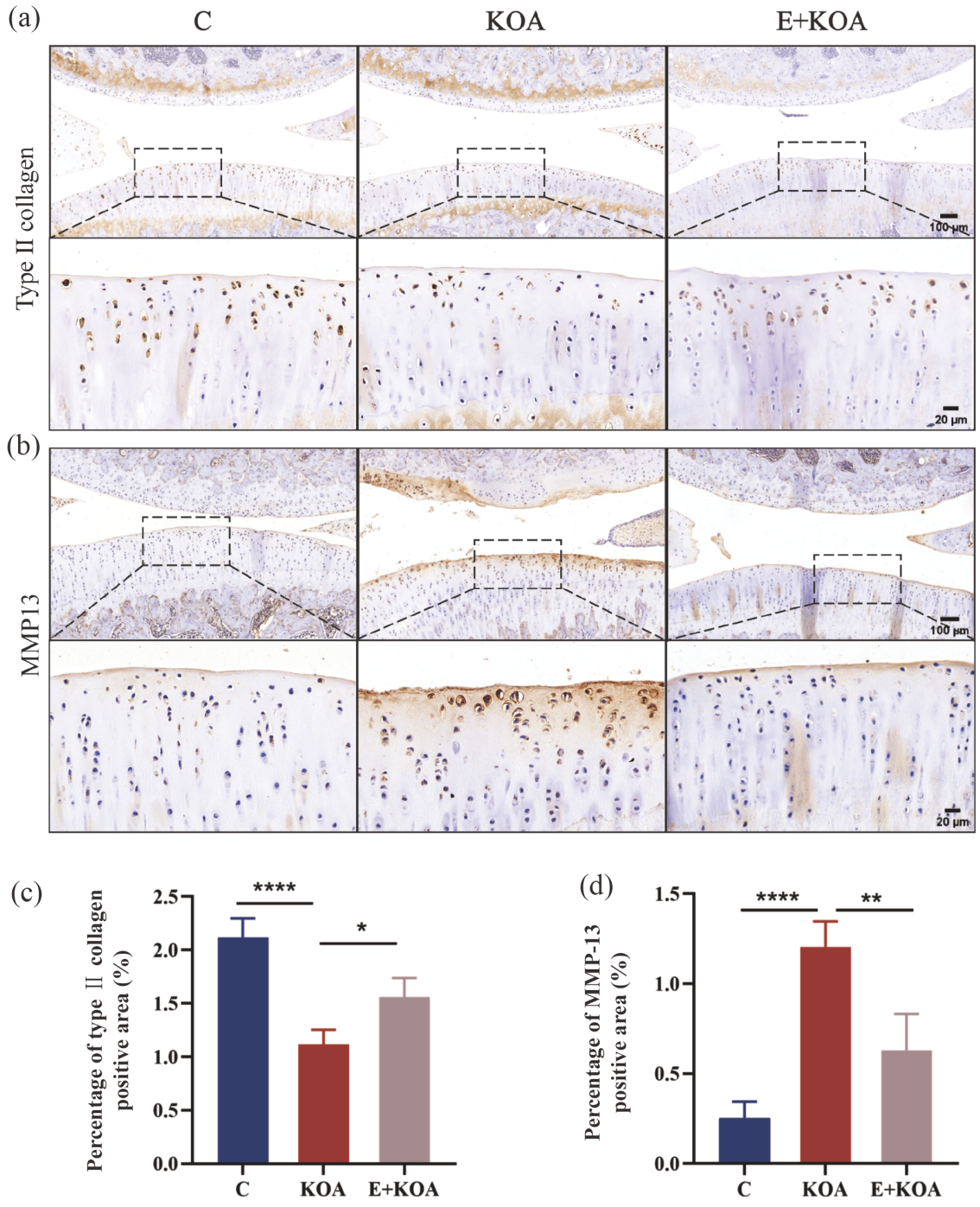

Figure 10 Staining of cartilage type II collagen and MMP-13: (a) staining of cartilage type II collagen; (b) staining of cartilage MMP-13; (c) area of cartilage type II collagen, positive percentage; (d) area of cartilage MMP-13 positive percentage.

degradation of cartilage matrix and promoting matrix synthesis.

4. Discussion

In recent years, researchers have improved their understanding of the pathogenesis of KOA, which is considered to be a chronic inflammatory disease primarily affecting articular cartilage and adjacent joint tissues. KOA involves various factors, including articular cartilage wear and tear, inflammatory response, alterations in joint fluids, bone alterations, and genetic factors, among other aspects [16]. Abnormal mechanical loading may cause impaired stability of the joint, leading to hypermobility or abnormal range of motion, which increases the risk of abnormal forces and

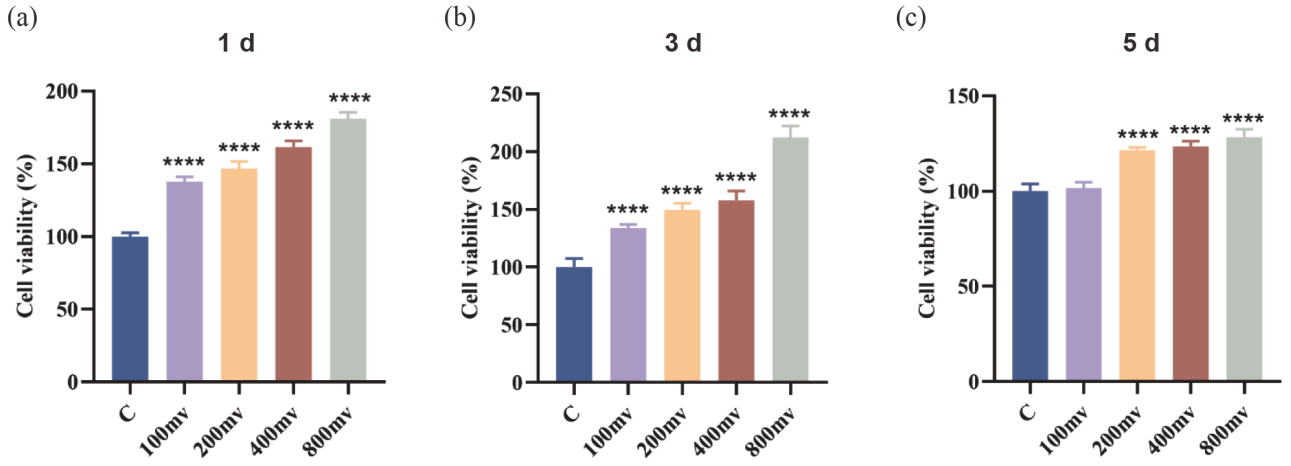

Figure 11 Chondrocyte viability: (a) chondrocyte viability at 1 d of treatment with different electrical stimulation intensities; (b) chondrocyte viability at 3 d of treatment with different electrical stimulation intensities; (c) chondrocyte viability at 5 d of treatment with different electrical stimulation intensities.

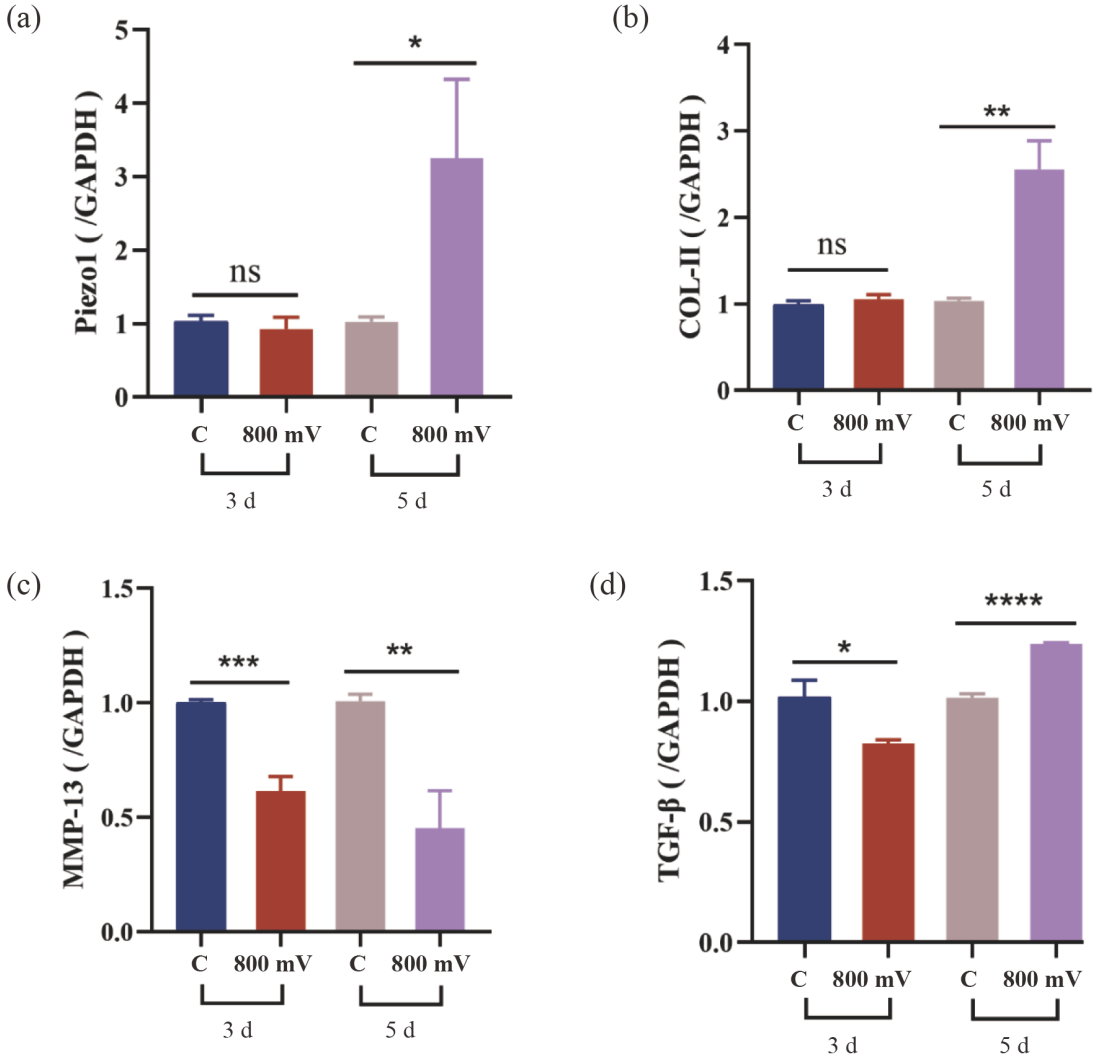

Figure 12 Effects of PES treatment for 3 or 5 d on the expression of Piezo1 and related marker genes in chondrocytes: (a) expression level of Piezo1 in chondrocytes treated with PES for 3 or 5 d; (b) expression level of type II collagen in chondrocytes treated with PES for 3 or 5 d; (c) expression level of MMP-13 in chondrocytes treated with PES for 3 or 5 d; (d) TGF-β expression level in chondrocytes treated with PES for 3 or 5 d.

stresses on the joint, further exacerbating cartilage wear and joint degeneration [17]. PES treats arthritis by applying pulses of electrical current at a specific frequency and intensity. It reduces pain caused by arthritis by stimulating nerve endings, interfering with the transmission of pain signals, promoting the activation of endogenous pain inhibitory systems, and reducing pain perception and transmission [18]. Studies have shown that electrical stimulation also improves blood supply to arthritis sufferers by stimulating vasodilation and increasing circulation, which aids in delivering oxygen and nutrients to joint tissues, promoting tissue repair and heal [19]. While these findings suggest that electrical stimulation has the potential to improve KOA, further studies are required to validate and explore the underlying mechanisms and effects in greater detail.

The rectus femoris and soleus are critical muscle groups in the human body, crucial for supporting and regulating lower limb movement. The rectus femoris is responsible for knee extension and hip flexion, and is one of the most important muscles in maintaining lower limb stability and facilitating movement. The rectus femoris is primarily responsible for knee extension, while the soleus is primarily responsible for knee flexion [20]. This coordinated contraction and relaxation allows the lower extremity to perform a variety of movements. Several studies have shown that the rectus femoris and soleus muscles undergo significant changes in patients with osteoarthritis, as evidenced by muscle atrophy, loss of muscle strength, changes in muscle tissue, and reduced function [21]. Therefore, in the treatment of osteoarthritis, it is also necessary to focus on the health of the surrounding muscles. In this study, we demonstrated a significant increase in the modulus of elasticity and a notable decrease in the mean cross-sectional area of muscle fibers in osteoarthritic flounder muscle through stretch testing and HE staining. This may be due to the structural changes in the muscle fibers of the soleus muscle due to osteoarthritis, where the muscle fibers become stiffer, leading to an increase in the elastic modulus of the muscle [22]. It has also been suggested that inflammation and damage caused by osteoarthritis may lead to fibrosis of the muscle tissue, with proliferation and accumulation of collagen fibers in the muscle tissue, making the muscle more stiff and rigid [23]. Importantly, we found that a 4-week PES intervention was able to significantly reduce the elastic modulus of the soleus muscle, with a significant increase in the mean cross-sectional area of muscle fibers. This suggests that PES attenuates the muscle fiber atrophy process and restores the normal elasticity of the muscle, thereby improving the functional status of the muscle. This is similar to the findings of Gonçalves et al. [24] and Jarecki et al. [25] by improving muscle atrophy, which leads to the treatment of osteoarthritis. The difference is that they used photobiomodulation therapy and physical activity. Electrical stimulation can stimulate muscle fibers, causing an increase in muscle contraction and vitality. By adjusting the parameters of frequency, intensity and pulse width of electrical stimulation, different degrees of muscle contraction effects can be produced, which can help to enhance the strength and stability of the muscles around the joints, and improve the function and motor control of the joints.

Articular cartilage is the smooth, tough tissue that sits on the surface of joint bones. It plays an important role in shock absorption, cushioning and protecting the joint. Chronic arthritis can cause degenerative and degenerative changes to the cartilage, and damage to the medial tibial plateau tends to be more severe than other areas. During normal walking and weight-bearing activities, the medial region of the knee is under more stress [26]. This imbalance in pressure distribution may lead to accelerated degeneration of the medial articular cartilage. Studies have found that patients with KOA have more severe joint space narrowing, degeneration of the medial tibial cartilage, and more pronounced medial knee pain and dysfunction [27]. Several studies have also found that medial cartilage degeneration and joint deformity are closely related to mechanical stress on the knee joint. Factors such as uneven force distribution, joint instability, abnormal joint lines of force, and overuse and injury may lead to excessive stress and injury to the medial cartilage, which in turn may lead to degeneration and deformation [28]. Hence, in this study, the medial tibia was chosen for subsequent experiments to more effectively evaluate the impact of PES on enhancing cartilage mechanics. The results showed a significant decrease in resistivity and compressive elastic modulus in the KOA group and a significant increase in cartilage resistivity and elastic modulus after PES intervention. The results of the electrical impedance test and compression test of cartilage had similar trends. Although cartilage resistivity and elastic modulus describe two different physical properties, they may be intrinsically related to each other, mainly in the organization and composition of cartilage. The network of collagen fibers in cartilage makes an important contribution to its mechanical stability and affects the elastic modulus [29]; the composition of these fiber networks and the intercellular matrix affects the movement of charge carriers, and hence resistivity [30]. Changes in resistivity and elastic modulus may be correlated during cartilage injury or degradation. Structural and compositional changes due to tissue degradation, such as breakage of collagen fibers and loss of proteoglycans, may both reduce the mechanical strength of cartilage (decreasing the elastic modulus) and may also decrease its resistivity. Therefore, the electrical properties of cartilage may be one of the useful parameters to predict the mechanical properties of cartilage. In this paper, compression tests and electrical impedance tests on cartilage revealed that PES effectively increased the elastic modulus of KOA cartilage

and helped to enhance the biomechanical properties of cartilage tissue.

The structure of cartilage in osteoarthritis undergoes multiple changes, including damage to the cartilage surface, dehydration and thinning of cartilage, fibrosis of cartilage, alterations in the repair of cartilage damage, and sclerosis of the subchondral bone [31]. These changes lead to a decrease in the elasticity and strength of cartilage, which affects the normal function of the joint. In order to analyze the effect of PES on the improvement of cartilage structure in osteoarthritis, the morphological characteristics of cartilage tissues were observed in the present study by tomato red solid green staining. The cartilage in the KOA group underwent obvious degradation, with the surface of articular cartilage being rough, the intensity of matrix staining being weakened, obvious fissures being seen, and the loss of chondrocytes, whereas in the electrical stimulation group the surface of the cartilage was smoother, with the structure being relatively intact, with the number of cells being slightly reduced, and matrix. The surface of cartilage in the electrical stimulation group was smoother, the structure was relatively intact, the number of cells was slightly reduced, and the matrix staining was uniform. This suggests that PES can promote the normal maintenance and repair of cartilage structure and positively affect the structure and function of cartilage. Zuzzi et al. [32] prepared cylindrical whole-layer cartilage defects *in vivo* and subjected them to current stimulation for 5 min. The proteoglycan content of the defects was significantly higher after 35 d of current stimulation. This suggests that microcurrent stimulation accelerates the repair process of non-articular hyaline cartilage. Both medication and physical therapy promote the repair and regeneration of cartilage to maintain its structural integrity and function. Unlike, however, the possible intrinsic connection between cartilage electrical impedance and elastic modulus in this study suggests that PES helps to enhance cartilage mechanical properties [33,34]. Type II collagen is a major component of cartilage tissue and is a key protein in maintaining the structure and function of cartilage tissue. It is a fibrillar collagen with a special three-dimensional structure that provides cartilage with elasticity and tensile strength [35]. MMP-13 mainly acts on type II collagen and is able to degrade and break down type II collagen molecules, destroying the integrity of the cartilage matrix. In diseases such as arthritis, overexpression of MMP-13 leads to cartilage destruction and degradation [36]. In order to further investigate the effect of PES on cartilage regeneration, the present study applied immunohistochemical staining to analyze the content of type II collagen and MMP-13 in cartilage. The results showed that the content of type II collagen in arthritic cartilage was significantly decreased and the content of MMP-13 was increased, which led to the destruction and degradation of cartilage matrix, accelerating the degenerative changes and functional impairment of the joints. However, PES inhibited the excessive activity of MMP-13 and promoted the synthesis and stabilization of type II collagen, which is important for the elasticity and tensile strength of cartilage as it provides a certain degree of flexibility and elasticity. This further confirms the positive effect of PES on the mechanical properties of cartilage tissue.

Chondrocyte viability is essential for the structure and function of cartilage. Chondrocytes are the main cell type of cartilage tissue, and they are responsible for synthesizing and maintaining the cartilage matrix, as well as participating in the repair and regeneration processes of cartilage [37]. Chondrocyte viability directly affects the physiological state and function of cartilage. Therefore, we selected 100, 200, 400, and 800 mV electrical stimulation to treat chondrocytes for 1, 3, and 5 d, respectively, and investigated the effects of different intensities of PES on chondrocyte viability. The findings revealed a significant increase in chondrocyte viability following 1, 3, or 5 d of electrical stimulation, with the most notable enhancement observed with 800 mV electrical stimulation. On this basis, we chose this parameter to investigate the effects of PES on the mRNA expression of Piezo1, collagen type II, MMP-13, and TGF-β in chondrocytes, which are mechanoreceptor channel proteins capable of sensing mechanical stimuli in the periphery of the cell. In cartilage tissues, Piezo1 can sense mechanical stimuli such as joint movements, gait changes, and external pressure, and thus activate intracellular signaling pathways to regulate cellular physiological functions [38]. It has been found that the expression and activity of Piezo1 are closely related to the proliferation and differentiation of chondrocytes, and the activation of Piezo1 can promote the proliferation and differentiation of chondrocytes by regulating the process of the cell cycle and promoting the expression of genes related to cell division and proliferation. The activation of Piezo1 can affect the synthesis and secretion of collagen, proteoglycans, and other matrix components by the chondrocytes, which can affect the synthesis of the cartilage matrix [39]. Piezo1 activation also affects the synthesis and secretion of matrix components such as collagen and proteoglycans by chondrocytes, thereby affecting the synthesis of cartilage matrix [40]. In addition, Piezo1 activation can inhibit the production of matrix-degrading enzymes, such as MMP-13, by chondrocytes, thus reducing the degradation of cartilage matrix [41]. In the present study, we initially found that PES significantly increased the expression of Piezo1 in chondrocytes, while the expression of type II collagen and TGF-β also increased significantly, and the expression of MMP-13 decreased significantly. This suggests that electrical stimulation may mimic the effect of mechanical stimulation and activate the intracellular signaling pathway, leading to an increase in the

expression of Piezo1, which may promote the synthesis of cartilage matrix by increasing the expression of type II collagen and TGF-β in chondrocytes. Nevertheless, more comprehensive studies on the specific mechanism and regulatory network of Piezo1 expression through electrical stimulation are required. This is not only crucial for uncovering the fundamental mechanism of cellular transformation in response to mechanical signals but also holds potential value for the development of therapeutic strategies related to electrical stimulation.

5. Conclusion

In this study, methods and techniques of experimental zoology and cell biology were used to establish a rat osteoarthritis model and investigate the effects of PES on the mechanical-biological properties of KOA joints. Additionally, *in vitro* experiments delved into the effects of electrical stimulation on the biological behavior of chondrocytes. The findings revealed the following outcomes: (1) PES effectively stimulates muscle fibers, increases the cross-sectional area of muscle fibers, prevents muscle atrophy and loss of function, and restores the mechanical properties of muscle tissue. These effects are crucial for sustaining joint stability and mitigating joint burden. (2) PES promotes the metabolic activity of chondrocytes, increases cartilage matrix synthesis, and improves the overall structure and mechanical properties of cartilage tissue. (3) PES increased the mRNA expression of Piezo1 in chondrocytes, and the elevation of Piezo1 may promote the increased expression of type II collagen and TGF-β, which in turn promotes the synthesis of cartilage matrix. Future studies may involve a detailed investigation of Piezo1-regulated signaling pathways by electrical stimulation to reveal the regulatory network of Piezo1 expression by electrical stimulation, which may provide a basis for the development of relevant therapeutic strategies and applications.

Conflict of interest *On behalf of all authors, the corresponding author states that there is no conflict of interest.*

Author contributions ***Yanru Xue:*** *Conceptualization, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing.* ***Zekun Hua:*** *Writing – review & editing.* ***Xinqi Lou:*** *Data curation, Writing – review & editing.* ***Yinuo Zhao:*** *Writing – review & editing.* ***Ying Shen:*** *Writing – review & editing.* ***Meng Zhang:*** *Writing – review & editing.* ***Haoyu Feng:*** *Validation, Writing – review & editing.* ***Xiaochun Wei:*** *Investigation, Writing – review & editing.* ***Yanqin Wang:*** *Writing – review & editing.* ***Xiaogang Wu:*** *Funding acquisition, Project administration, Resources, Supervision, Writing – review & editing.* ***Weiyi Chen:*** *Supervision, Writing – review & editing.*

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脉冲电刺激对膝骨关节炎生物力学影响的实验研究

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摘要 骨关节炎是最常见的关节疾病之一, 导致患者关节疼痛、功能障碍、生活质量下降, 因此, 探索更有效的预防和治疗的方法去缓解患者疼痛, 提高生活质量尤为重要. 在物理治疗中, 脉冲电刺激(PES)因其安全性高、操作简便等特点, 被认为是一种很有前景的治疗方法, 为患者提供了一种非侵入性、安全有效的选择. 但目前, 关于PES对关节周围组织的生物力学影响的研究较少, 其对软骨细胞生物学行为的影响也尚不明确. 本研究探讨了PES对膝骨关节炎关节生物力学性能和软骨细胞生物学行为的影响. 结果表明, 强度为10 mA、频率为4 Hz的PES可增加肌纤维的横截面积, 防止肌肉萎缩和功能丧失, 恢复肌肉组织的力学性能. PES还能有效增加膝骨关节炎(KOA)软骨的电阻率, 提高软骨的弹性模量, 从而增强软骨的生物力学特性. PES还能促进软骨细胞的代谢活性, 增加软骨基质的合成, 从而改善软骨的整体结构和力学性能. 另外, 细胞实验研究表明, 连续5天的800 mVPES可显著提高软骨细胞中Piezo1基因的表达水平, 同时使II型胶原和TGF-β的表达增加, 而MMP-13的表达减少, 这些变化有利于促进软骨基质的合成. 这对于保护和改善关节健康, 减轻膝骨关节炎的影响有着积极的作用, 对于理解PES对软骨细胞的作用机制和制定相关的治疗策略具有重要意义.