Stem Cell Research & Therapy

RESEARCH

Open Access

Effect of traction therapy on muscle satellite cell proliferation and differentiation in a rat model of knee stiffness

Xiaoqian Lv[1], Xiaoyu Wang[2], Jianxing Yue[1], Xin Wang[2], Haoyue Chen[2] and Qiang Gao[2*]

Abstract

Background In the rat knee stiffness model, the duration of traction treatment is mostly 20–40 min; however, relatively few studies have been conducted on longer traction treatment of extended knee stiffness in rats. Therefore, the aim of this study was to explore the efficacy of prolonged traction and its mechanism of action in extended knee stiffness in rats.

Methods The model of extended knee joint stiffness was established in rats and treated with powered flexion position traction. On the 10th and 20th days respectively, passive range of motion (PROM) assessments and musculoskeletal ultrasound were conducted. Rectus femoris muscle tissues were taken for Western blotting (WB) to detect the expression of muscle satellite cells proliferation and differentiation signaling factors. Histopathological staining was used to evaluate the degree of muscle atrophy and muscle fibrosis in the rectus femoris muscle, and immunofluorescence double staining was used to detect proliferation of muscle satellite cells number. The results from these analyses were used to assess the therapeutic outcomes of the traction treatment.

Results The findings indicated that chronic persistent traction significantly improved joint mobility, notably enhanced the proliferation of muscle satellite cells, and inhibited their differentiation. Furthermore, the treatment facilitated the repair and regeneration of damaged tissues, reduced muscular atrophy and fibrosis in the rectus femoris muscle, and alleviated knee stiffness.

Conclusion Chronic persistent traction can effectively relieve knee joint stiffness, and its mechanism is related to the activation and proliferation of the rectus femoris muscle satellite cells, thereby promoting the repair and regeneration of damaged skeletal muscle.

Keywords Extended knee stiffness, Traction, Muscle satellite cells, Muscle regeneration, Muscle fibrosis

*Correspondence:
Qiang Gao
314483462@qq.com
[1]The Second Affiliated Hospital of Bengbu Medical University, Bengbu 233040, China
[2]The Affiliated Taian City Central Hospital of Qingdao University, Taian 271000, China

Introduction

Knee stiffness is a common complication of trauma, knee osteoarthritis, knee replacement, neurological disorders (such as spinal cord injury, stroke, cerebral palsy, etc.), and joint immobilization [1–5]. Once it occurs, if not treated by an orthopedic or rehabilitation professional, it can dramatically reduce the patient's quality of life and even lead to lifelong disability [6]. Clinically, the treatment of knee stiffness is divided into conservative treatment and surgical treatment, of which traction treatment is an effective conservative method [7]. In traction therapy, factors such as the traction angle, weight, and duration all influence the therapeutic outcome. Traction can be categorized into rapid and chronic traction based on the duration of application. Prior studies predominantly selected rapid traction, typically lasting 20–40 min, and have demonstrated that 30 min traction yields superior efficacy [8–10]. Chronic persistent traction adheres to the law of tension-stress (LTS), as proposed by Ilizarov. This principle states that biological tissues can stimulate cell growth and tissue regeneration through continuous, stable, and gradual traction. Consequently, it plays a crucial role in the repair of limb defects. By regulating the traction force and duration, it is possible to regenerate damaged bones and soft tissues. This process is also known as distraction histogenesis (DH) [11]. In terms of traction weight, two ways of low load and high load can be selected, studies have proved that compared with high-load, short-term traction, low-load, long-term traction produces more mechanical energy, so that the tissue can be elongated to a greater extent, and the treatment effect of joint stiffness is more significant [12, 13]. Muscle satellite cells, which are considered to be skeletal muscle adult stem cells, possess a strong ability to continuously self-renewal as well as proliferative differentiation [14]. The division of muscle satellite cells after activation follows the stem cell division pattern, that is, two types of daughter cells are generated after cell division, one of which remains in its original state as the source of future cell division, and the other can further differentiate and develop into mature muscle fibers. Under normal circumstances, muscle satellite cells are in a resting state and do not divide and proliferate. But when local myofibroblasts sense external stimulation, which causes the release of locally relevant stimulus signaling factors, muscle satellite cells are activated and begin to undergo large-scale cell division and proliferation, and then transfer to the site of the injured skeletal muscle, which then forms new myoblasts and promotes repair function of them [15, 16]. Muscle satellite cells related signaling factors such as myogenin (MyoG) is involved in the regulation of muscle satellite cells differentiation, myogenic factor 5 (Myf5), paired box 7 (Pax7) and myoblast determination protein 1 (MyoD1) are involved in the regulation of muscle satellite cells activation and proliferation, and these signaling factors enable the interaction and signaling between muscle satellite cells and myofibers. It has been demonstrated that mechanical pulling inhibits skeletal muscle satellite cells differentiation and promotes muscle satellite cells activation and proliferation [17]. Mechanical stimulation promotes the proliferative capacity of muscle satellite cells, and the proliferative capacity of the cells is related to the magnitude of the load of the stimulus to which the cells are subjected [18]. Therefore, we hypothesized that under the stimulation of chronic persistent traction, muscle satellite cells are activated and proliferated, thereby improving the structure of muscles around the knee joint and alleviating knee joint stiffness.

Therefore, this study established the rat model of extended knee joint stiffness and used the method of chronic persistent traction to investigate its therapeutic effect on rat extended knee joint stiffness, and provided theoretical basis for future clinical application.

Materials and methods

Laboratory animals

The work has been reported in line with the ARRIVE guidelines 2.0. Sixty-four adult male Sprague Dawley (SD) rats, approximately 8 weeks of age, were came from Jinan Pengyue Laboratory Animal Breeding Co., Ltd., China. The rat experiments were approved by the Laboratory Animal Ethics Committee of the Affiliated Taian City Central Hospital of Qingdao University (NO.2024-07-02). According to different traction times, randomly split the rats into group N (normal group, without any intervention, $n=16$), group M (model group, without treatment after modeling, $n=16$), group R (regular traction group, traction time 0.5 h/times, $n=16$), and group C (chronic persistent traction group, traction time 1.5 h/times, $n=16$) by using the random number table method.

Experimental design

Modeling methods: Except for the normal group, all other groups used the polymer fixation bandage braking method to construct an extended knee stiffness model in rats (Fig. 1A). Using a small animal anesthesia machine (SA428, Jiangsu, China), rats were subjected to general anesthesia with inhaled isoflurane for the establishment of knee joint stiffness model. The knee joint of one side of the rats was completely straightened and immobilized from the pelvis to the proximal part of the ankle, with the ankle plantarflexed at about 60 degrees. The rat knee joint extended stiffness model was established by using polymer fixed bandage for 6 weeks. The rats were kept in an experimental environment of 20–25 °C, 12 h light-dark cycle, and routinely provided with food and water. During the experimental period, except for the immobilized side of the knee joint, the rest of the limbs

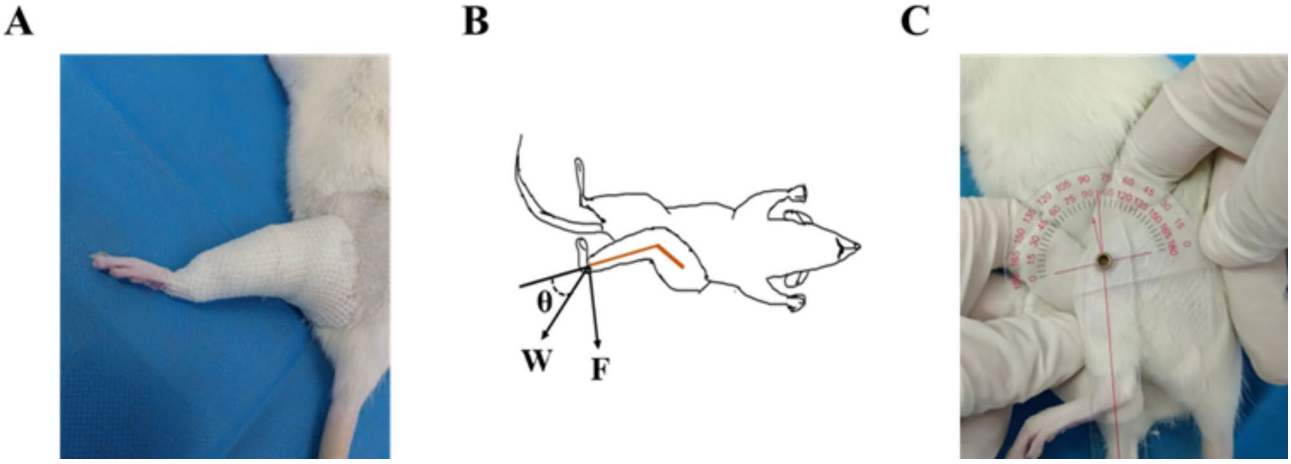

Fig. 1 (**A**) A rat model of extended knee stiffness was constructed by applying a polymer immobilization bandage to immobilize one side of the knee for 6 weeks. (**B**) The model rat was subjected to traction treatment with a traction torque of 0.02 N·m, when θ was 90 degrees, that is under the condition of a traction force of 0.4 N. (**C**) Measurement of knee joint PROM in rat

of the rats were not restricted. The rats did not receive any surgical intervention and analgesics. After the rat model was completed, the range of motion of the model rat was measured to ensure the success of the knee stiffness model.

Traction method: Rats were anesthetized by inhalation of isoflurane during traction. The rats were first rapidly anesthetized in the pre-anesthesia box of the small animal anesthesia machine, and then placed on the operating table to continuously anesthetize with a mask with a continuous anesthesia concentration range of 1.5-2%, and subjected to traction treatment. The traction treatment was performed once a day for 10 and 20 days. In addition to the normal group and the model group, the rats in the treatment group received traction treatment, and the polymer bandage was removed and not used during the whole period of traction treatment, and the range of motion of rats in each group was assessed on the animals prior to the model, under knee stiffness induction and after treatment. During the traction treatment, the femur part of the rats was kept immobile, and continuous traction treatment was given in the direction of knee flexion. The force of traction should not be too large to avoid causing secondary injury to the tissues around the joint. The traction force was derived from according to $T = W \cdot L \cdot \sin\theta$ (where W is the magnitude of the force of traction, θ is the angle between the direction of traction and the tibia, L is the length of the tibia of the rat, and T is the torque in N·m). Traction was performed under the conditions of torque of 0.02 N·m and θ of 90 degrees, with the direction of traction perpendicular to the rat tibia and the point of traction stopping at the very end of the rat tibia, and the length of the rat tibia was measured to be 0.05 m. At this time, the W traction force was 0.4 N, and the value of F was therefore 0.4 N [19] (Fig. 1B).

Passive range of motion (PROM) measurement: After anesthesia, the rats were placed on an operating table, the femur and knee joints of rats were fixed, and the center of the lateral side of the knee joint was taken as the axis, and the prolongation line of the longitudinal axis of the femur towards the distal end was taken as 0 degree, and the angle between this line and the tibia was measured by a protractor to measure the joint mobility of the rats (measured three times, and the average value was taken), which was completed by the same professional trained researchers who did not participate in the present study (Fig. 1C).

The rats were euthanized, in line with the national guidelines for euthanasia of laboratory animals (GB/T 39760−2021), and after 10 and 20 days of traction treatment, the rats were first inhaled with excessive isoflurane anesthetic, and then confirmed dead. The rectus femoris muscle was dissected and isolated, and used for WB, Hematoxylin-eosin (HE) staining, Masson's trichrome staining, and immunofluorescence staining for detection.

WB analysis

SD rats rectus femoris muscle samples were extracted, and tissue lysates (Western and IP cell lysates, Beyotime, P0013, China) were prepared. The mixtures were homogenized in a low-temperature tissue homogenizer (Jingxin, Tissuelyser-24, China) 2 times for 1 min each time, The supernatant was collected by centrifugaation at 12,000 X rpm at 4℃ for 20 min after continued cracking on the smoothie from the ice machine (TOUHE, XB-100, China) for 30 min. Protein concentration was determined using the BCA protein concentration assay kit (Solarbio, PC0020, China). Protein lysates were separated on a 12% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) (Solarbio, PE005, China) and transferred onto a polyvinylidene fluoride (PVDF) membrane

(Merck Millipore, Ireland). The following antibodies were applied: rabbit anti-MyoG (Affinity, DF8273, China; dilution 1:2000), rabbit anti-Myf5 (Affinity, DF3089, China; dilution 1:1000), rabbit anti-Pax7 (Affinity, AF7584, China; dilution 1:1000), rabbit antibody against MyoD1 (Affinity, AF7733, China; dilution 1:1000), GAPDH (Servicebio, GB15002, China; dilution 1:1000) and goat anti-rabbit IgG-HRP (ZSGB-BIO, ZB2301, China; dilution 1:5000). The membranes were then evaluated using an enhanced chemiluminescence system (BIO.RAD, USA) according to the manufacturer's instructions. Protein band density was quantified using Image J software (National Institutes of Health, USA).

HE staining

Rectus femoris muscle samples were removed from 4% paraformaldehyde, and then paraffin embedding, sectioning, and finally HE staining. The CKX53 inverted microscope with 200 magnification (Olympus, Japan) was used to capture the cross-sectional area (CSA) of rectus femoris muscle of rats, and the CSA was measured with ImageJ software (National Institutes of Health, USA). Each group randomly selected 6 visual fields for analysis.

Masson staining

Rectus femoris muscle specimens were embedded, paraffin sectioned, sections were treated by Masson's staining solution. Microscopic examination was performed, and the ratio of collagen fiber area to muscle fiber area was measured using ImageJ software (National Institutes of Health, USA), and six fields of view were randomly chosen by each group for analysis.

Immunofluorescence double staining

Rectus femoris muscle tissues were paraffin sectioned, for immunofluorescence double staining. The primary antibodies were mouse anti-Desmin primary antibody (Servicebio, GB12075, China; dilution 1:200) and rabbit anti-Ki-67 (Servicebio, GB111141, China; dilution 1: 200). The secondary antibodies were Alexa Fluor 488 goat anti-mouse (Servicebio, GB25301, China; dilution 1:400) and CY3 goat anti-rabbit (Servicebio, GB21303, China; dilution 1:300). Each section was observed with a fluorescence microscope (Olympus, Japan) at 400 times magnification. Image acquisition was performed by the same experimenter who was unaware of the grouping, and six fields of view were randomly selected for analysis in each group.

Ultrasound shear wave elastography

Ultrasound shear wave elastography was used to obtain elasticity indices in rat rectus femoris muscle [20, 21]. Ultrasound shear wave elastography was obtained using a color ultrasound Doppler system (Resona R9, China) equipped with the L14-3WU linear transducer. After the rats in each group were anesthetized, the rat hairs on the affected limb was removed cleanly and then placed on the operating table for ultrasound detection. The electron linear array probe coated with ultrasonic coupler was placed on each muscle abdomen of the rectus femoris muscle of the rat for longitudinal detection. The skeletal muscle shear modulus (G, kPa) and shear wave propagation velocity (V, m/s) were directly obtained from the ultrasound elastography analysis software. The ultrasonic shear wave elastic imaging examinations involved in the experiment were all completed by the same doctor who was not aware of the experimental group and had more than 10 years of professional training and ultrasound diagnosis experience, in order to avoid causing experimental errors.

Statistical methods

Data analysis and statistical plotting were performed using GraphPad Prism 8.0.2 software. The data were presented as mean ± standard deviation. Measurement data were first tested for normality, when they conformed to normal distribution, paired t-tests were selected for within-group comparison, one-way ANOVA was used for comparison between multiple groups, and Tukey test was chosen specifically for post hoc comparison. When not conforming to normal distribution, the nonparametric rank sum test (such as the Mann-Whitney test) was selected. $P<0.05$ was considered statistically significant.

Results

Improvement of knee joint PROM in rats by traction therapy

PROM of rats in each group was measured before and after traction treatment. Before modeling, no significant difference were observed in PROM across the four groups ($P>0.05$). Before traction treatment, there was no significant difference in PROM between groups M, R, and C ($P>0.05$), but compared to group N, there was a significant decrease in PROM between the above three groups (VS group N_{10}, $M_{10}P<0.0001$, $R_{10}P<0.0001$,$C_{10}P<0.0001$; VS group N_{20}, $M_{20}P<0.0001$, $R_{20}P<0.0001$, $C_{20}P<0.0001$). This indicated that after 6 weeks of knee immobilization, PROM was significantly limited and knee stiffness was induced.

After traction treatment, the measurement results of rat knee joint PROM (Fig. 2), we found that the PROM was significantly reduced in groups M, R, and C compared with group N ($P<0.05$); and the joint mobility was significantly increased in groups, R, and C compared with group M (VS group M_{10}, $R_{10}P<0.0001$,$C_{10}P<0.0001$; VS group M_{20}, $R_{20}P<0.0001$, $C_{20}P<0.0001$), with a significant increase in group C compared with group R (VS group R_{10}, $C_{10}P=0.0490$; VS group R_{20}, $C_{20}P=0.0005$).

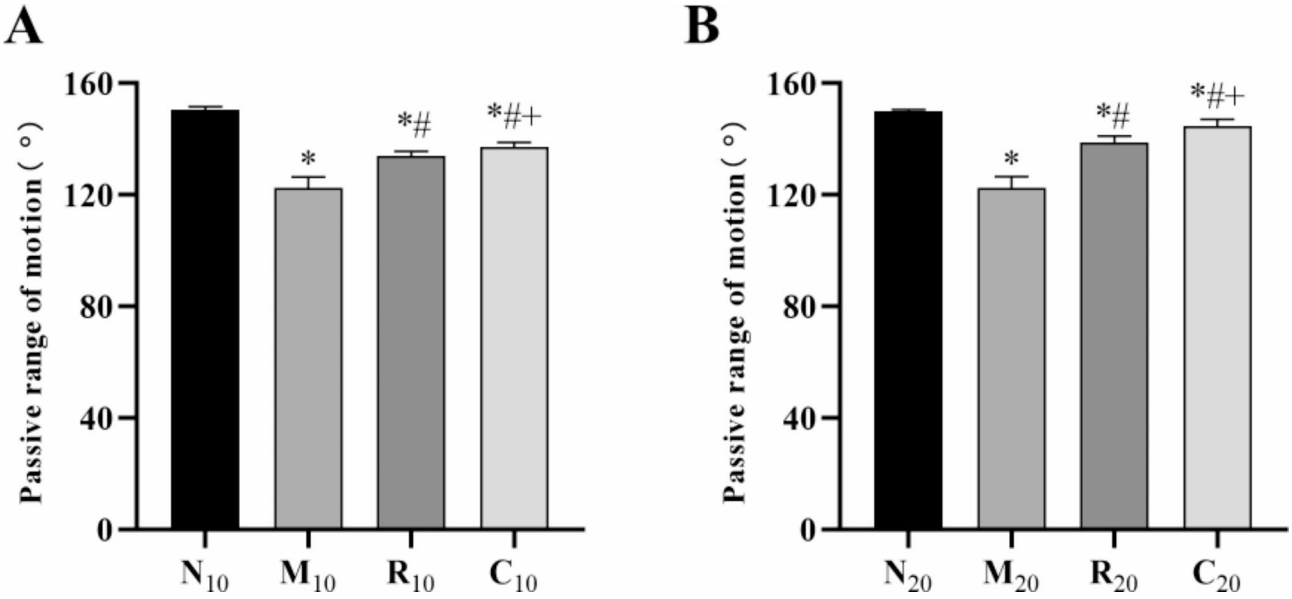

Fig. 2 Results of rats knee joint PROM in each group. (**A**, **B**) The results of knee joint motion measurements in rats at 10 and 20 days of traction treatment, respectively ($n=8$). $^{*}P<0.05$ versus group N. $^{\#}P<0.05$ versus group M. $^{+}P<0.05$ versus group R

Therefore, in both 10 and 20 days traction treatment, the knee joint mobility of rats could be improved, and when the traction time was 1.5 h/time, the chronic persistent traction group improved the joint motion more significantly than the normal group, effectively relieving the knee joint stiffness.

Effect of traction on proliferation and differentiation of muscle satellite cells

Muscle satellite cells are a class of pluripotent stem cells found in muscle tissue, and the regulatory factor Pax7 and myogenic regulatory factors (MRFs), such as MyoD1, Myf5, and MyoG, are involved in the regulation of muscle satellite cells, which leads to proliferation and differentiation, and thus regulation of muscle development and regeneration [22, 23]. In the results of protein expression levels of MyoD1, Pax7, Myf5 and MyoG in rectus femoris muscle in each group after traction treatment (Fig. 3). We conclude that the model rats exhibited a reduced proliferative capacity of muscle satellite cells, coupled with an increased level of their differentiation. Both treatment groups reduced the differentiation level of muscle satellite cells, but the chronic persistent traction group significantly enhanced the proliferation of satellite cells, thereby promoting muscle regeneration.

To further confirm the effect of chronic persistent traction on muscle satellite cell proliferation, we performed immunofluorescence double staining experiments on rat rectus femoris muscle.

According to the results of fluorescence staining (Fig. 4), the number of proliferation of muscle satellite cells in the rectus femoris muscle in the group M was the least, and even no proliferation occurred. The number of muscle satellite cell proliferation increased in both treatment groups, but the proliferation was most significant in the chronic persistent traction group (VS group R_{10}, $C_{10}P=0.0002$; VS group R_{20}, $C_{20}P<0.0001$), when the muscle satellite cells proliferation state was optimal, which could effectively promote the repair and regeneration of injured skeletal muscle, contributing to the alleviation of knee joint stiffness.

Effect of traction on muscle atrophy and fibrosis in rectus femoris muscle

The following experiments were conducted to investigate the effects of traction therapy on muscle tissue. In the results of HE staining and Masson staining (Fig. 5), we clearly found that the rectus femoris muscle CSA was the smallest and the ratio of collagen fiber area to muscle fiber area was the largest in group M, which indicated that the rectus femoris muscle was the most severely atrophied and fibrotic in the rats knee stiffness model. The CSA of rectus femoris muscle fibers increased significantly in both treatment groups after 10 and 20 days of traction treatment, and increased more in the chronic persistent traction group, which could better improve the muscle atrophy. The decrease in collagen fiber area to muscle fiber area ratio was lower in both treatment groups, and the percentage of collagen deposition was the smallest in the chronic persistent traction group, which could significantly reduce the fibrosis of the rectus femoris muscle.

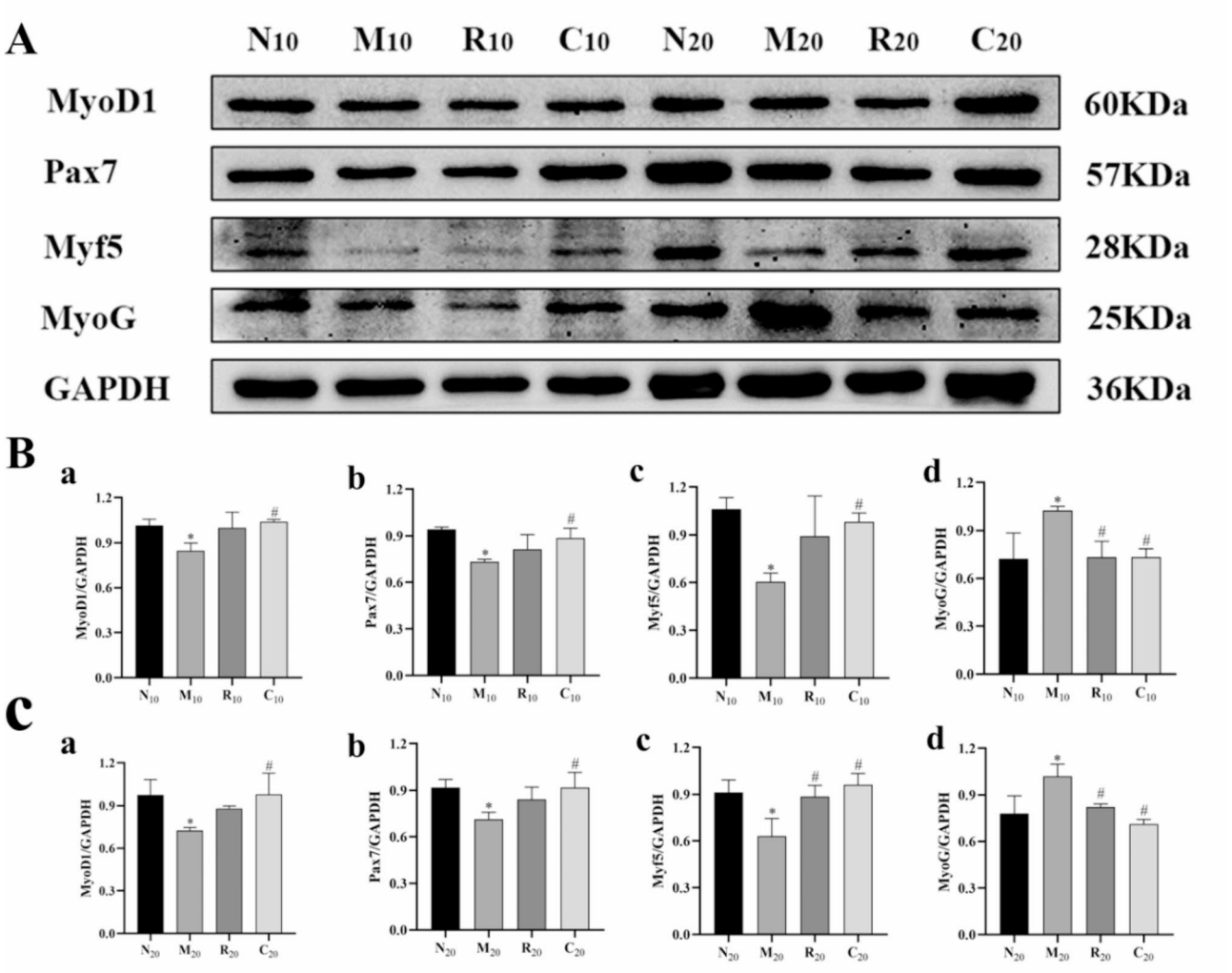

Fig. 3 Results of protein expression levels of MyoD1, Pax7, Myf5 and MyoG. (**A**) Western blot of MyoG, Myf5, Pax7, MyoD1, and GAPDH. (**B**, **C**) Quantitative results of protein expression in rectus femoris muscle of each group on 10 and 20 days of traction treatment, respectively ($n=8$). $^{*}P<0.05$ versus group N. $^{\#}P<0.05$ versus group M. $^{+}P<0.05$ versus group R. Full-length blots are presented in Supplementary Fig. 1

We performed musculoskeletal ultrasonography to further explore the effect of chronic persistent traction on the elasticity of the rectus femoris muscle.

The results of ultrasound shear wave elasticity imaging of the rectus femoris muscle in each group after 10 and 20 days of traction treatment (Fig. 6). Among the groups, the shear modulus and shear wave propagation velocity of the rectus femoris muscle in group M were the largest, and the larger they were, the smaller the tissue elasticity, so we can conclude that the least elasticity and the most serious injury in the knee stiffness model rats. After 10 and 20 days of traction treatment, the shear modulus and shear wave propagation velocity of the rectus femoris muscle were significantly reduced in both treatment groups, indicating that traction treatment can improve the elasticity of the rectus femoris muscle. We also found that the shear modulus and shear wave propagation velocity were the smallest in the chronic persistent traction group, so improving the elasticity of the damaged rectus femoris muscle plays a significant therapeutic role.

Discussion

Knee stiffness is defined as limited active and passive joint motion of the knee joint, accompanied by deformity, pain, etc., fibrosis and shortening of the joint capsule and soft tissues around the joint, limited ductility or increased stiffness [24]. The most common cause of joint stiffness is joint immobilization [6, 25]. The terms stiffness and contracture are also used to describe limited PROM in the knee joint [26]. In experimental animal models, joint contractures induced by immobilization within 2 weeks are primarily myogenic and the contractures are reversible. When immobilized for 4 weeks or longer, the resulting joint contracture is mixed, including articular and myogenic contracture [27]. Prolonged joint

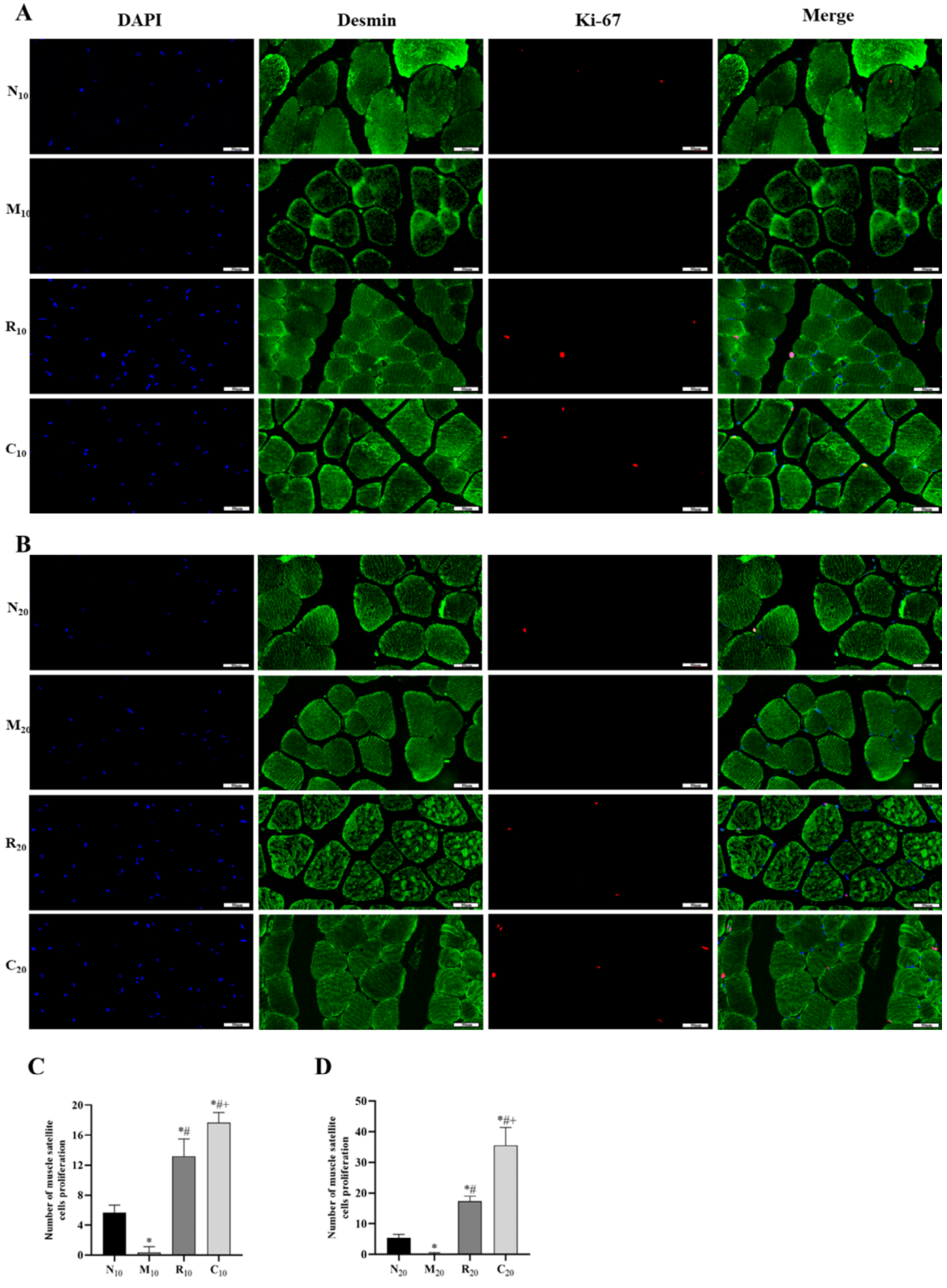

Fig. 4 Results of immunofluorescence double staining of rectus femoris muscle in rats. (**A**, **B**) Representative photographs of immunofluorescence double-stained sections of rectus femoris muscle in each group (DAPI-labeled nuclei were in blue color, Desmin-labeled muscle satellite cells are in green color, and Ki-67-labeled cells were in red color for cell proliferation, and Desmin and Ki-67 were co-expressed as the number of proliferating muscle satellite cells, scale bar = 50 μm). (C, D) The number of muscle satellite cell proliferation in immunofluorescence double-stained sections of rectus femoris muscle of rats in each group after 10 and 20 days of traction treatment ($n = 6$). As the staining was performed on harvested tissue, it is possible that more cellular proliferation would have been present in vivo. $^{*}P < 0.05$ versus group N. $^{\#}P < 0.05$ versus group M. $^{+}P < 0.05$ versus group R

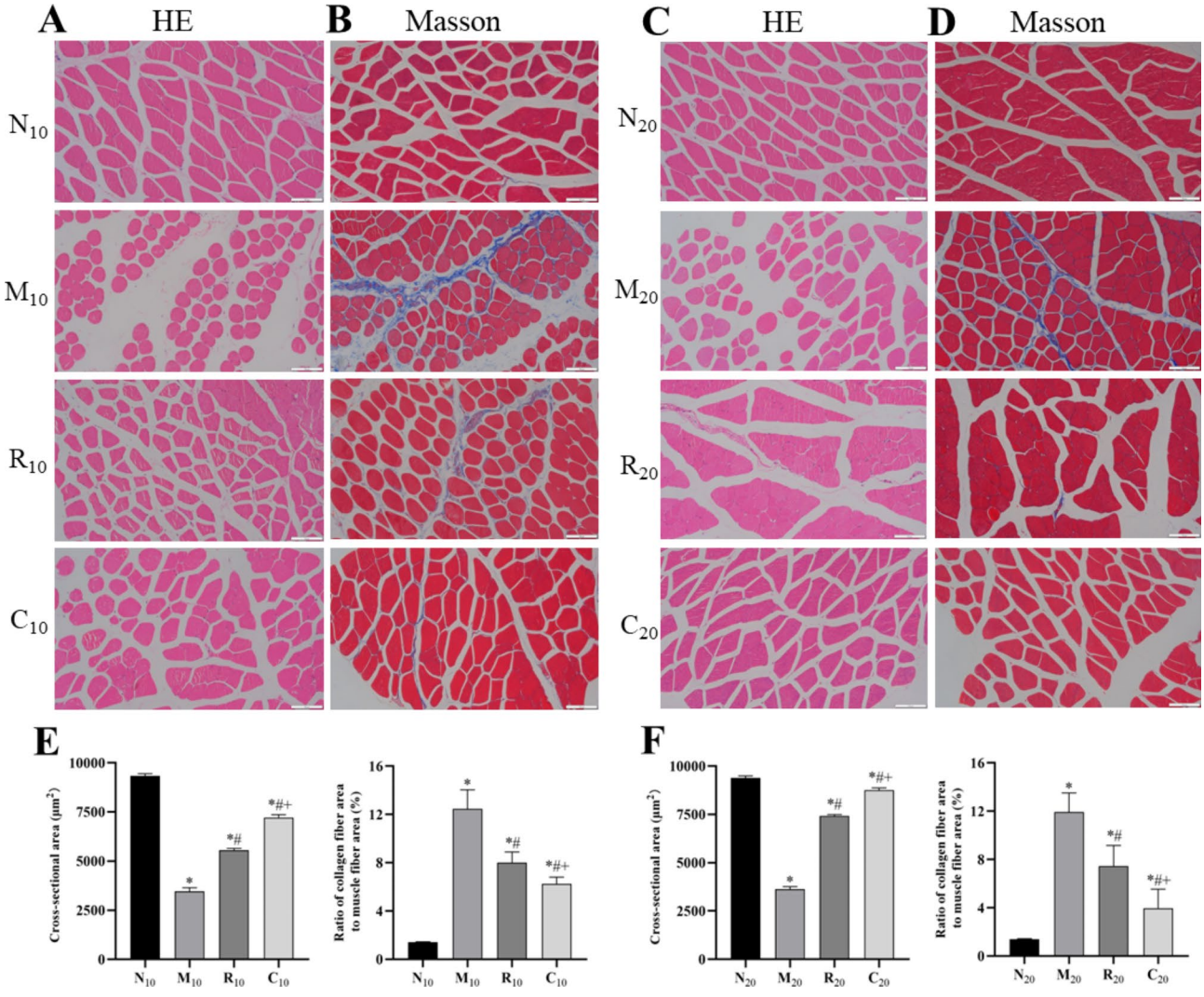

Fig. 5 Results of HE staining and Masson staining of rat rectus femoris muscle. (**A**-**D**) Representative photographs of HE stained and Masson stained sections of rectus femoris muscle of rats in each group (scale bar = 200 μm). Nuclei were blue and cytoplasm was red in HE staining; collagen fibers were blue in Masson staining; myofibrils, fibrillar pigment and erythrocytes were red. (**E**, **F**) The results of CSA of myofibers in HE stained sections and the ratio of collagen fiber area to muscle fiber area in Masson stained sections were counted after 10 and 20 days (areas of collagen fiber deposition were indicated in blue, $n = 6$). $^{*}P < 0.05$ versus group N. $^{\#}P < 0.05$ versus group M. $^{+}P < 0.05$ versus group R

braking beyond 4 weeks should be avoided and to prevent irreversible joint contractures, which will limit the PROM of the joint more and make treatment more difficult [28]. For joint stiffness caused by prolonged immobilization, the pathological changes include fibrosis of the joint capsule and pathological changes in the muscles and ligaments, and although there is currently more research on the joint capsule in joint stiffness [29–31], clinical treatment should not only improve the fibrosis of the joint capsule, but also focus on the treatment of myogenic factors. The focus on myogenic factors is also because it appears early and reversible, with the implementation of the concept of accelerated rehabilitation surgery, more and more attention is paid to early rehabilitation, so the myogenic factors affecting the joint stiffness are more significant to the treatment. The present experiment focuses on the changes in the muscle organization of the rectus femoris muscle in knee stiffness with traction therapy.

Stress relaxation loads utilize low-intensity forces to position the joint at its terminal joint range of motion and maintain the joint tissue at its maximum therapeutic length [32]. As the tissue lengthens as a result of the applied stress, adjustments are made during traction to position the joint tissue at its new maximum therapeutic length, which increases joint range of motion [33]. This stretching and holding process is repeated several times during the treatment and is performed once a day to increase tissue elasticity and achieve plastic deformation to lengthen the soft tissue [34]. In a study comparing

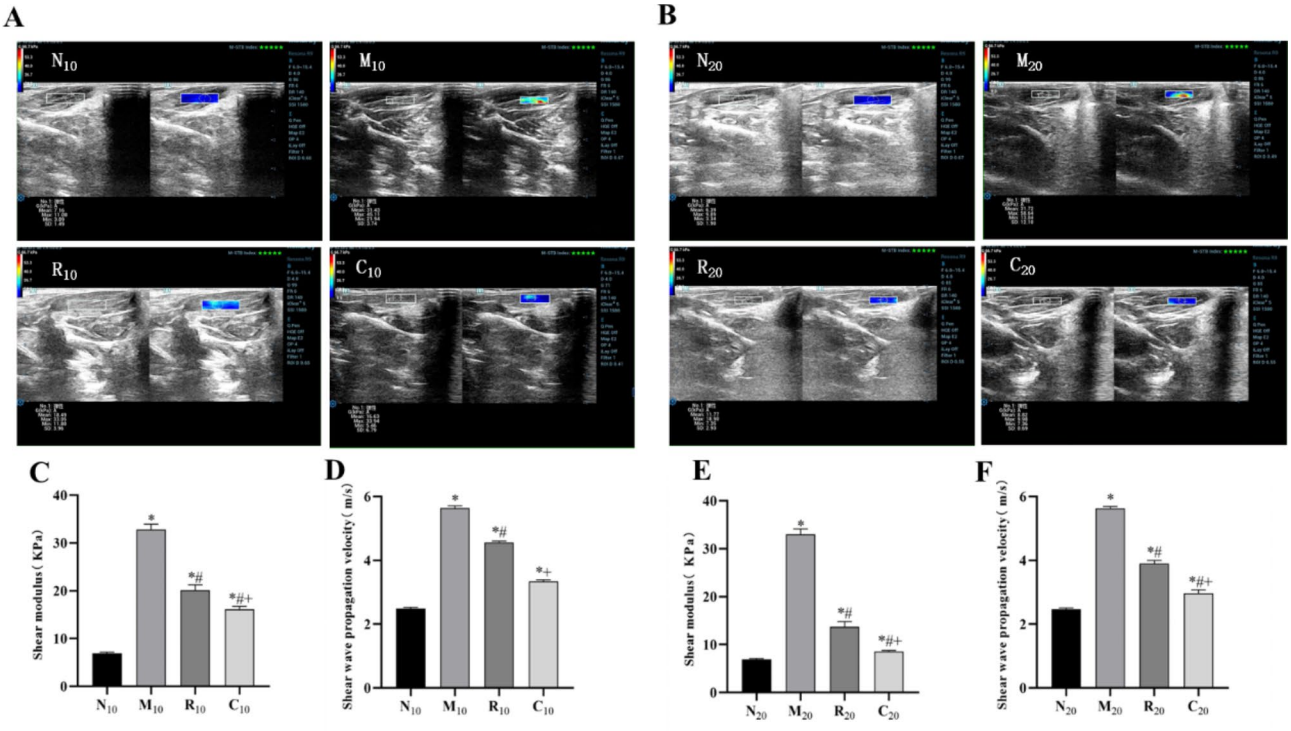

Fig. 6 Ultrasound shear wave elastography of the rectus femoris muscle in rats. (**A**, **B**) Representative images of ultrasound shear wave elastography of each group the rectus femoris muscle. (**C**) and (**E**) were the shear modulus results on elastography at 10 and 20 days of traction treatment, respectively. (**D**) and (**F**) were the results of shear wave propagation velocity in the elastography in 10 and 20 days of treatment, respectively ($n=6$). $^{*}P<0.05$ versus group N. $^{\#}P<0.05$ versus group M. $^{+}P<0.05$ versus group R

variable torque stretching and duration, rat knees were subjected to repetitive stretching with different torques and durations after 40 days of immobilization, and only in the low-torque-long stretch group was there a significant improvement in deformation and failure load, better recovery of joint motion and more normal mechanical properties [19]. Immobilization reduces the longitudinal muscle tension, which is the basis of muscle contracture [35], so tension is necessary to maintain and restore the mechanical properties of muscle tissue; conversely, a reduced state of tension (e.g., produced by immobilization) weakens mechanical properties and promotes myogenic contracture [36–39]. After fixation induced knee joint stiffness in rats, the rectus femoris muscle atrophies and knee joint motion significantly decreases [40]. We found that animal models of flexion joint stiffness are more frequently studied [3, 27, 36, 41, 42], however, one of the signs that distinguish us humans from other mammals is upright walking, and the knee joint is in a functional position in order to bear weight and carry out flexion and extension activities of the lower limbs, so clinically the knee joint is fixed in the knee extension position after knee arthroplasty, and therefore the extension type of knee joint stiffness is most common. The time of traction treatment is often chosen 20–40 min, and studies have shown that traction for 30 min is more effective in improving joint motion [43]. Therefore, in this experiment, we used low load, long time traction therapy, once a day, to further explore the therapeutic effect of chronic persistent traction on knee joint stiffness.

In the experimental results, we observed that the knee joint PROM of model rats was significantly limited, severe atrophy and fibrosis of the rectus femoris muscle, reduced activation and proliferation of muscle satellite cells and significant differentiation, and the rectus femoris was seriously injured. Compared with normal traction treatment, chronic persistent traction treatment effectively improved knee joint PROM of rats, significantly increased the CSA of rectus femoris muscle fibers, effectively reduced the ratio of collagen fiber to muscle fiber area of rectus muscle, as well as the shear modulus and shear wave propagation velocity, which played a good therapeutic role in improving the atrophy and fibrosis of rectus femoris muscle. Moreover, the chronic persistent traction group could significantly increase the proliferation of rectus femoris muscle satellite cells, which was even far more than that of normal rats, so the chronic persistent traction could activate and proliferate the muscle satellite cells, which can effectively promote the better repair and regeneration of damaged skeletal muscle, and improve the knee joint stiffness.

While the final experimental results were obtained in this study, the potential limitations of translating the results of the rat model into human treatment were also recognized. In the experiment, the rats were treated with traction under anesthesia, while the patients with knee stiffness in the clinic were awake throughout the treatment, and they were able to perceive the comfort or discomfort of the treatment due to changes in the traction treatment program such as traction time or traction weight, as well as feedback on the results of traction treatment, so that our clinicians or therapists could adjust the treatment plan at any time, but this was not reflected in the experimental rats. Clinically, the course and severity of patients with knee stiffness are not consistent, which requires us to develop a personalized treatment plan.

Conclusion

In this study, we found that prolonged traction effectively relieves knee stiffness, and the mechanism is related to the fact that chronic persistent traction promotes the activation and proliferation of rectus femoris muscle satellite cells, which effectively facilitates the repair and regeneration of skeletal muscle.

At present, there is no unified consensus on the traction duration and weight of traction treatment, and the therapeutic effect of different traction weights at different times is not completely clear, so future research needs to further clarify the interaction between the two to provide a theoretical basis for the selection of clinical treatment regimens.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13287-024-04108-1.

Supplementary Material 1

Acknowledgements
Not applicable.

Author contributions
XQL designed experiments, constructed animal stiffness model and writing-Original draft preparation. XYW and JXY performed most of experiment. XW and HYC collected and analyzed the experimental data. QG designed experiments and revised the manuscript. All authors have approved the final manuscript.

Funding
This research was funded primarily by the Shandong Provincial Traditional Chinese Medicine Science and Technology Project (M-2022081).

Data availability
The date used and analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
The animal experiment was approved by the Animal Ethics Committee of the Affiliated Taian City Central Hospital of Qingdao University (Approval number: 2024-07-02. Title of the approved project: Based on the "meridian tendon theory", the mechanism of chronic persistent traction on extended knee stiffness in rats. Date of Approval: May 30, 2022). The rats were euthanized, in line with the national guidelines for euthanasia of laboratory animals (GB/T 39760 – 2021).

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Received: 20 September 2024 / Accepted: 6 December 2024
Published online: 20 December 2024

References

1. Wegner E, Slotina E, Mickan T, Truffel S, Arand C, Wagner D, et al. Pleiotropic long-term effects of atorvastatin on posttraumatic joint contracture in a rat model. Pharmaceutics. 2022;14(3):523. https://doi.org/10.3390/pharmaceutics14030523
2. Yan D, Yang J, Pei F. Total knee arthroplasty treatment of rheumatoid arthritis with severe versus moderate flexion contracture. J Orthop Surg Res. 2013;8:41. https://doi.org/10.1186/1749-799x-8-41
3. Sasabe R, Sakamoto J, Goto K, Honda Y, Kataoka H, Nakano J, et al. Effects of joint immobilization on changes in myofibroblasts and collagen in the rat knee contracture model. J Orthop Res. 2017;35(9):1998–2006. https://doi.org/10.1002/jor.23498
4. Birnkrant DJ, Bushby K, Bann CM, Apkon SD, Blackwell A, Brumbaugh D, et al. Diagnosis and management of Duchenne muscular dystrophy, part 1: diagnosis, and neuromuscular, rehabilitation, endocrine, and gastrointestinal and nutritional management. Lancet Neurol. 2018;17(3):251–67. https://doi.org/10.1016/s1474-4422(18)30024-3
5. Zaffagnini S, Di Paolo S, Meena A, Alesi D, Zinno R, Barone G, et al. Causes of stiffness after total knee arthroplasty: a systematic review. Int Orthop. 2021;45(8):1983–99. https://doi.org/10.1007/s00264-021-05023-3
6. Mao D, Mi J, Pan X, Li F, Rui Y. Suppression of TGF-beta activity with remobilization attenuates immobilization-induced joint contracture in rats. Injury. 2021;52(3):434–42. https://doi.org/10.1016/j.injury.2020.12.029
7. Haffar A, Goh GS, Fillingham YA, Torchia MT, Lonner JH. Treatment of arthrofibrosis and stiffness after total knee arthroplasty: an updated review of the literature. Int Orthop. 2022;46(6):1253–79. https://doi.org/10.1007/s00264-022-05344-x
8. Wang L, Cui JB, Xie HM, Zuo XQ, He JL, Jia ZS, et al. Effects of different static progressive stretching durations on range of motion, myofibroblasts, and collagen in a posttraumatic knee contracture rat model. Phys Ther. 2022;102(5). https://doi.org/10.1093/ptj/pzab300
9. Chen K, Zhang X, Ren K, Liu H, Liao YY, Wen CH, et al. Effect and mechanism of static progressive extension with different parameters on knee joint stiffness in rats. Chin J Orthop Trauma. 2024;26(3):255–61. https://doi.org/10.3760/cma.j.cn115530-20240105-00011
10. Tian HY, Zuo XQ, Yin SS, Jia ZS, Zhang LN, Xie HM et al. Effect of static progressive draft with different duration on traumatic knee contracture in rats. Chin J Emerg Resusc Disaster Med. 2021,16(4):427–30.
11. Zhu YL, Guo BF, Zang JC, Pan Q, Zhang DW, Peng Y, et al. Ilizarov technology in China: a historic review of thirty-one years. Int Orthop. 2022;46(3):661–8. https://doi.org/10.1007/s00264-021-05238-4
12. Light KE, Nuzik S, Personius W, Barstrom A. Low-load prolonged stretch vs. high-load brief stretch in treating knee contractures. Phys Ther. 1984;64(3):330–3. https://doi.org/10.1093/ptj/64.3.330
13. MacKay-Lyons M. Low-load, prolonged stretch in treatment of elbow flexion contractures secondary to head trauma: a case report. Phys Ther. 1989;69(4):292–6. https://doi.org/10.1093/ptj/69.4.292
14. Tatsumi R. Mechano-biology of skeletal muscle hypertrophy and regeneration: possible mechanism of stretch-induced activation of resident myogenic

stem cells. Anim Sci J. 2010;81(1):11–20. https://doi.org/10.1111/j.1740-0929.2009.00712.x
15. Berardi E, Annibali D, Cassano M, Crippa S, Sampaolesi M. Molecular and cell-based therapies for muscle degenerations: a road under construction. Front Physiol. 2014;5:119. https://doi.org/10.3389/fphys.2014.00119
16. Dumont NA, Bentzinger CF, Sincennes MC, Rudnicki MA. Satellite cells and skeletal muscle regeneration. Compr Physiol. 2015;5(3):1027–59. https://doi.org/10.1002/cphy.c140068
17. Kook SH, Son YO, Choi KC, Lee HJ, Chung WT, Hwang IH, et al. Cyclic mechanical stress suppresses myogenic differentiation of adult bovine satellite cells through activation of extracellular signal-regulated kinase. Mol Cell Biochem. 2008;309(1–2):133–41. https://doi.org/10.1007/s11010-007-9651-y
18. Lindsay A, Larson AA, Verma M, Ervasti JM, Lowe DA. Isometric resistance training increases strength and alters histopathology of dystrophin-deficient mouse skeletal muscle. J Appl Physiol (1985). 2019;126(2):363–75. https://doi.org/10.1152/japplphysiol.00948.2018
19. Usuba M, Akai M, Shirasaki Y, Miyakawa S. Experimental joint contracture correction with low torque–long duration repeated stretching. Clin Orthop Relat Res. 2007;456:70–8. https://doi.org/10.1097/BLO.0b013e31803212bf
20. Sun L, An S, Zhang Z, Zhou Y, Yu Y, Ma Z, et al. Molecular and metabolic mechanism of low-intensity pulsed ultrasound improving muscle atrophy in hindlimb unloading rats. Int J Mol Sci. 2021;2222. https://doi.org/10.3390/ijms222212112
21. Wang F, Zhou CX, Zheng Z, Li DJ, Li W, Zhou Y. Metformin reduces myogenic contracture and myofibrosis induced by rat knee joint immobilization via AMPK-mediated inhibition of TGF-β1/Smad signaling pathway. Connect Tissue Res. 2023;64(1):26–39. https://doi.org/10.1080/03008207.2022.2088365
22. Hao R, Wang Y, Chen L, Liu Y, Zhang W. Differences in inferior oblique muscle satellite cell populations between primary and secondary inferior oblique muscle overaction. Ophthalmic Res. 2023;66(1):301–6. https://doi.org/10.1159/000527742
23. Tian ZL, Wang RL, Yang QF, Qin ZQ, Dong HW, Zou DH, et al. Detection of multiple biomarkers associated with satellite cell fate in the contused skeletal muscle of rats for wound age estimation. Int J Legal Med. 2023;137(3):875–86. https://doi.org/10.1007/s00414-023-02971-w
24. Sung EJ, Chun MH, Hong JY, Do KH. Effects of a resting foot splint in early brain injury patients. Ann Rehabil Med. 2016;40(1):135–41. https://doi.org/10.5535/arm.2016.40.1.135
25. Kaneguchi A, Ozawa J, Kawamata S, Yamaoka K. Development of arthrogenic joint contracture as a result of pathological changes in remobilized rat knees. J Orthop Res. 2017;35(7):1414–23. https://doi.org/10.1002/jor.23419
26. Zhou H, Trudel G, Goudreau L, Laneuville O. Knee joint stiffness following immobilization and remobilization: A study in the rat model. J Biomech. 2020;99:109471. https://doi.org/10.1016/j.jbiomech.2019.109471
27. Trudel G, Laneuville O, Coletta E, Goudreau L, Uhthoff HK. Quantitative and temporal differential recovery of articular and muscular limitations of knee joint contractures; results in a rat model. J Appl Physiol (1985). 2014;117(7):730–7. https://doi.org/10.1152/japplphysiol.00409.2014
28. Born CT, Gil JA, Goodman AD. Joint contractures resulting from prolonged immobilization: etiology, prevention, and management. J Am Acad Orthop Surg. 2017;25(2):110–6. https://doi.org/10.5435/jaaos-d-15-00697
29. Dagneaux L, Limberg AK, Owen AR, Bettencourt JW, Dudakovic A, Bayram B, et al. Knee immobilization reproduces key arthrofibrotic phenotypes in mice. Bone Joint Res. 2023;12(1):58–71. https://doi.org/10.1302/2046-3758.121.Bjr-2022-0250.R2
30. Ando A, Suda H, Hagiwara Y, Onoda Y, Chimoto E, Itoi E. Remobilization does not restore immobilization-induced adhesion of capsule and restricted joint motion in rat knee joints. Tohoku J Exp Med. 2012;227(1):13–22. https://doi.org/10.1620/tjem.227.13
31. Wong K, Trudel G, Laneuville O. Intra-articular collagenase injection increases range of motion in a rat knee flexion contracture model. Drug Des Devel Ther. 2018;12:15–24. https://doi.org/10.2147/dddt.S144602
32. McHugh MP, Magnusson SP, Gleim GW, Nicholas JA. Viscoelastic stress relaxation in human skeletal muscle. Med Sci Sports Exerc. 1992;24(12):1375–82.
33. Cyr LM, Ross RG. How controlled stress affects healing tissues. J Hand Ther. 1998;11(2):125–30. https://doi.org/10.1016/s0894-1130(98)80009-4
34. Bhave A, Sodhi N, Anis HK, Ehiorobo JO, Mont MA. Static progressive stretch orthosis-consensus modality to treat knee stiffness-rationale and literature review. Ann Transl Med. 2019;7(Suppl 7):S256. https://doi.org/10.21037/atm.2019.06.55
35. Tinklenberg J, Beatka M, Bain JLW, Siebers EM, Meng H, Pearsall RS, et al. Use of ankle immobilization in evaluating treatments to promote longitudinal muscle growth in mice. Muscle Nerve. 2018;58(5):718–25. https://doi.org/10.1002/mus.26296
36. Kaneguchi A, Ozawa J, Minamimoto K, Yamaoka K. Morphological and biomechanical adaptations of skeletal muscle in the recovery phase after immobilization in a rat. Clin Biomech (Bristol Avon). 2020;75:104992. https://doi.org/10.1016/j.clinbiomech.2020.104992
37. Palmes D, Spiegel HU, Schneider TO, Langer M, Stratmann U, Budny T, et al. Achilles tendon healing: long-term biomechanical effects of postoperative mobilization and immobilization in a new mouse model. J Orthop Res. 2002;20(5):939–46. https://doi.org/10.1016/s0736-0266(02)00032-3
38. Ohira T, Kawano F, Ohira T, Goto K, Ohira Y. Responses of skeletal muscles to gravitational unloading and/or reloading. J Physiol Sci. 2015;65(4):293–310. https://doi.org/10.1007/s12576-015-0375-6
39. Pang KT, Loo LSW, Chia S, Ong FYT, Yu H, Walsh I. Insight into muscle stem cell regeneration and mechanobiology. Stem Cell Res Ther. 2023;14(1):129. https://doi.org/10.1186/s13287-023-03363-y
40. Ji LL, Yeo D. Cellular mechanism of immobilization-induced muscle atrophy: a mini review. Sports Med Health Sci. 2019;1(1):19–23. https://doi.org/10.1016/j.smhs.2019.08.004
41. Hagiwara Y, Ando A, Onoda Y, Matsui H, Chimoto E, Suda H, et al. Expression patterns of collagen types I and III in the capsule of a rat knee contracture model. J Orthop Res. 2010;28(3):315–21. https://doi.org/10.1002/jor.20997
42. Ozawa J, Kaneguchi A, Tanaka R, Kito N, Moriyama H. Cyclooxygenase-2 inhibitor celecoxib attenuates joint contracture following immobilization in rat knees. BMC Musculoskelet Disord. 2016;17(1):446. https://doi.org/10.1186/s12891-016-1303-5
43. Moriyama H, Tobimatsu Y, Ozawa J, Kito N, Tanaka R. Amount of torque and duration of stretching affects correction of knee contracture in a rat model of spinal cord injury. Clin Orthop Relat Res. 2013;471(11):3626–36. https://doi.org/10.1007/s11999-013-3196-z

Publisher's note