ORIGINAL ARTICLES

The effects of radiofrequency hyperthermia on type II collagen formation in the osteoarthritic knee

Zhaohua Fu*[1], Jiaojiao Zhao[2], Fei Zhang[3], Yongfei Wu[1], Qingqing Cao[2], Jiao Kong[1], Chunjuan Zhou[2]

[1] *Department of Rehabilitation Medicine, The Third Affiliated Hospital of Inner Mongolia Medical University, Baotou, China*
[2] *Inner Mongolia Medical University, Baotou, China*
[3] *Department of Orthopaedics, The Third Affiliated Hospital of Inner Mongolia Medical University, Baotou, China*

Received: May 12, 2018 **Accepted:** June 28, 2018 **Online Published:** September 10, 2018
DOI: 10.14725/dcc.v5n3p1 **URL:** http://dx.doi.org/10.14725/dcc.v5n3p1

Abstract

Objective: To explore the effect of radiofrequency hyperthermia on type II collagen expression in a rabbit model of osteoarthritis (OA).
Methods: Experimental model of knee OA was replicated in the right hind limbs of 54 male rabbits by using modified Hulth modeling method. The rabbits were randomly divided into Model group, Lugua Polypeptide group and Radiofrequency Hyperthermia group. After modeling, Lugua Polypeptide group was given intramuscular injection of Lugua polypeptide; Radiofrequency Hyperthermia group was treated with radiofrequency hyperthermia; Model group was not given any special treatment. On the 7^{th}, 13^{th} and 19^{th} day after radiofrequency hyperthermia, six experimental rabbits were chosen from each group and sacrificed to take out medial femoral condyle cartilages in the right hind limbs. Modified Mankins rating was applied to the morphological evaluation. Meanwhile, quantitative real-time PCR was used to detect the content of type II collagen in cartilage tissues of medial femoral condyle.
Results: At each time point after treatment, Mankins scores were decreased in all the 3 groups, with that of Model group significantly higher than those of the other two groups (Model group > Lugua Polypeptide group > Radiofrequency Hyperthermia group). The contents of type II collagen in cartilage tissues were increased in all the 3 groups, with that of Radiofrequency Hyperthermia group significantly higher than those of the other two group (Model group < Lugua Polypeptide group < Radiofrequency Hyperthermia group). The difference between groups was of statistical significance ($p < .05$). For Radiofrequency Hyperthermia group, Mankins scores were decreased gradually as the treatment time went by, with the content of type II collagen in cartilage tissues increased. The difference between time points was of statistical significance ($p < .05$).
Conclusions: Radiofrequency hyperthermia is superior to Lugua polypeptide in the treatment of knee OA, at least in rabbits. Its therapeutic mechanism may be related to the significant increase in type II collagen in cartilages.

Key Words: Knee osteoarthritis, Radiofrequency hyperthermia, Type II collagen, Rabbits

Osteoarthritis (OA) is a chronic degenerative joint disease characterized by articular cartilage degeneration and secondary hypertrophic osteoarthropathy. The main feature of the disease is the progressive loss of articular cartilages.[1] Collagen is an important component of the extracellular matrix, in which type II collagen accounts for 80% to 95% of the total collagen content. The change in quality and quantity is the direct cause of the loss of normal biomechanical properties of articular cartilages.[2,3] At present, the clinical treatment of knee OA is intractable due to the lack of effec-

*Correspondence: Zhaohua Fu; E-mail: fuzhaohua66@163.com; Address: Department of Rehabilitation Medicine, The Third Affiliated Hospital of Inner Mongolia Medical University, Baotou, China.

tive therapies. Radiofrequency hyperthermia that has been gradually developed in recent years has many advantages. In clinical application, it has been found that this technology can significantly relieve the symptoms of patients with knee OA, but the exact treatment mechanism remains unknown.[4] As a clinically used drug for knee OA, Lugua polypeptide has the function of promoting blood circulation for removing blood stasis and collaterals, improving blood flow in the subchondral medullary cavity and promoting the removal of metabolic wastes, which is of great value in protecting articular cartilage tissues.[5] In this study, after establishing the rabbit knee OA model, radiofrequency hyperthermia or Lugua polypeptide was used to give an interventional treatment, in order to compare the effects of radiofrequency hyperthermia and Lugua polypeptide on the morphology of rabbit knee articular cartilages and the content of type II collagen in articular cartilages, with the aim of exploring the relevant mechanism of radiofrequency hyperthermia for the treatment of knee OA.

1 Materials and methods

1.1 Main experimental materials

The experimental animals in this study were 54 male rabbits in total, provided by Beijing Tongzhou Experimental Animal Breeding Factory (Animal Certification: 2000 No. 013 Clean Grade). Each of the experimental rabbits weighed (2.50 ± 0.25) kg, 6 months old, and they were fed and kept separately in Laboratory Animal Center of the Third Affiliated Hospital of Inner Mongolia Medical University. The main experimental materials of this study also included rabbit experimental tables, hemostatic forceps, needle holders, curved needles, hooks, holders, blades, wire scissors, ophthalmic scissors, sutures, electronic balances, bone knives, etc.; the main experimental equipments included HG-2000 type in-vitro high frequency hyperthermia apparatus, GNP-9080 incubator, CMl850 freezing microtome, PPTHK-21B water bath, 7500 real-time quantitative PCR instrument, etc.; the main experimental drugs included Lugua polypeptide for injection, Sumianxin II, gentamycin sulfate injection, 10% ethylene diamine tetraacetic acid (EDTA), xylene, 0.1 M phosphate buffered solution (PBS), 4% paraformaldehyde, anhydrous ethanol, hematoxylin, type II collagenase, quantitative real-time PCR assay kit etc.

1.2 Rabbit knee OA modeling

All the experimental rabbits were fed and kept separately in Laboratory Animal Center of the Third Affiliated Hospital of Inner Mongolia Medical University. The breeding room was kept dry, ventilated and quiet, with suitable temperature and humidity. The size of the cage can ensure the free activities of the experimental rabbits. Breeding cages were required to be cleaned up daily, along with periodical UV disinfection. Meanwhile, it was necessary to closely observe the experimental rabbits for their food intake, activities, stools and urines. Before the formal experiment could proceed, the modified Hulth method[5] which was commonly used at home and abroad was applied to the modeling pre-experiment in this study. Four experimental rabbits were selected, and then their anterior and posterior cruciate ligaments, medial collateral ligaments and medial meniscuses of knee joints were surgically cut off (see Figure 1), causing knee joints unstable. Meanwhile, the knee joint that was turned outward was turned into an inversion, which changed the biomechanical axis of the knee joint, leading to the changes in the stress of articular cartilages accordingly. After the operation, the rabbits were fed and kept separately, with each rabbit in a single cage and free activities. The knee joint was reopened surgically in 6 weeks after the first operation. The success criteria of knee OA modeling were as follows: the synovial part of the knee joint showed nodular hyperplasia, the synovial fluid was in a small amount and turbid, with the formation of the surface ulcers, fissures, even some osteophytes.[6] It was found that all the experimental rabbits in the pre-experiment were successfully modeled (see Figure 2). In the formal experiment, the healthy experimental rabbits after 1 week of feeding were applied to the knee OA modeling by use of the modified Hulth modeling method. After achieving satisfactory anesthesia outcomes, touched the inside of the right hind limb of the rabbit to locate the joint space. A longitudinal incision with a length of about 3 cm was made along the midpoint of the space. Subsequently, the medial collateral ligament was cut off and the articular capsule was opened, with the medial meniscus freed and removed by a sharp knife along the space between the meniscus and the tibial plateau, so that the anterior cruciate ligament was exposed and cut off. After being washed with normal saline, the right hind limb was disinfected again with povidone-iodine, sutured and bound up. The model rabbits were divided into Radiofrequency Hyperthermia group, Lugua Polypeptide group and Model group by use of the random number table method, with 18 experimental rabbits in each group.

1.3 Intervention after modeling

In Radiofrequency Hyperthermia group, the experimental rabbits were given radiofrequency hyperthermia provided by HG-2000 in-vitro hyperthermia apparatus made in Zhuhai. Two circular electrodes (about 20 cm in diameter) of the in-vitro hyperthermia apparatus were placed on the upper and the lower part of the knee joint of the right hind limb of the experimental rabbit, with the distance between the electrode and the skin ranging from 15 cm to 20 cm. The output power percentage was 10%-20%, with the resonance mode selected and the temperature set to 36.5°C-38.5°C. The above-mentioned radiofrequency hyperthermia

ISSN 2375-8449 E-ISSN 2375-8473

was performed for 10 minutes, once a day, 6 times for one course of treatment, and the experimental rabbits were allowed to rest for 3 days after the end of each course. For Lugua Polypeptide group, the experimental rabbits were given intramuscular injection of Lugua polypeptide (manufactured by Heilongjiang Dilong Pharmaceutical Co., Ltd., 8 mg, powder, dissolved in 4 ml of sterile water for injection according to the instructions) following the dosage of 0.1 ml/kg, once a day. In Model group, the experimental rabbits were given no special intervention after modeling and placed in the cage for free activities. Both Radiofrequency Hyperthermia group and Lugua Polypeptide group were given intervention daily at a set period of time to ensure the regularity of treatment.

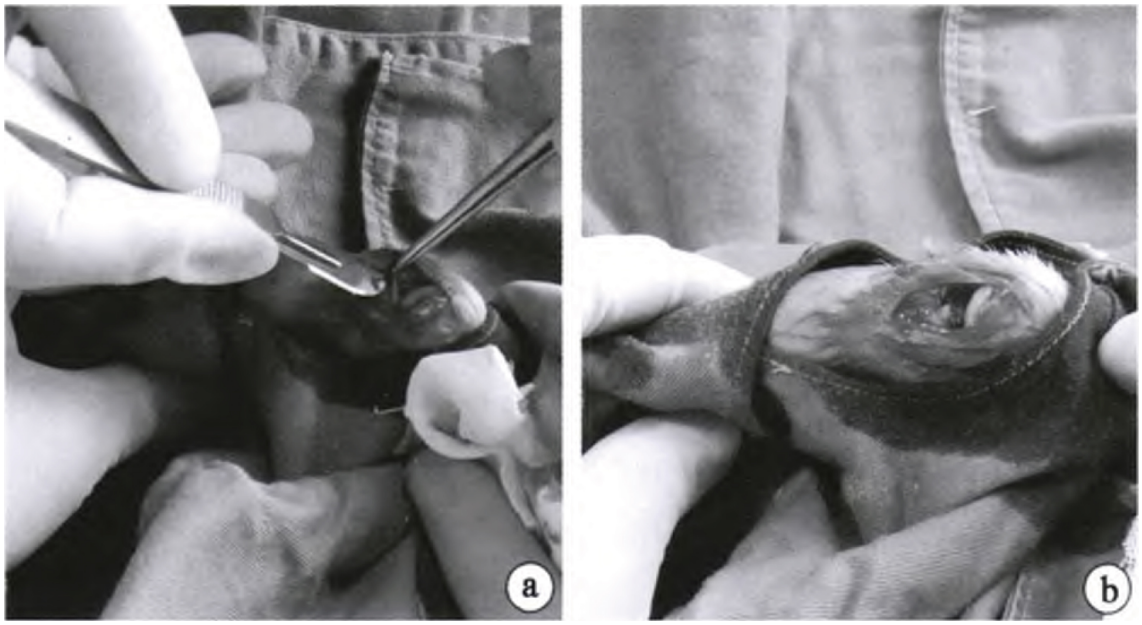

Figure 1: Keys to OA modeling
Note. a. To touch the inside of the right hind limb to locate the joint space, along which a longitudinal incision with a length of about 3 cm was made, with the medial collateral ligament cut off subsequently; b. To open the articular capsule, remove the medial meniscus, expose and cut off the anterior cruciate ligament.

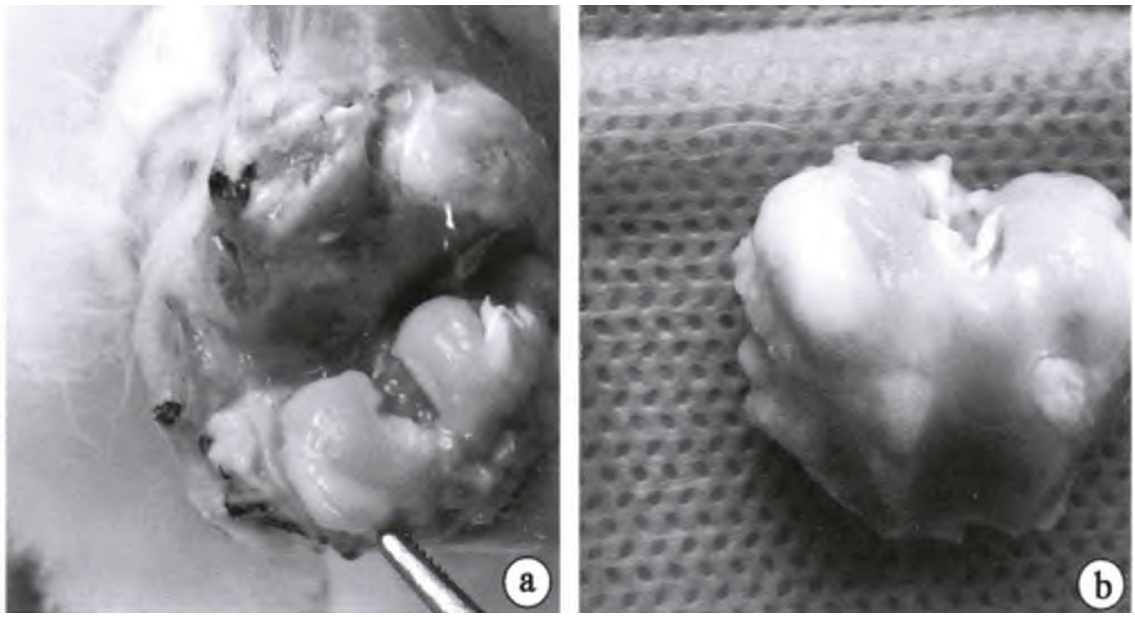

Figure 2: Success criteria of OA modeling
Note. It was visible that the synovial part of the knee joint showed nodular hyperplasia, even with the formation of some osteophytes.

1.4 Specimen making and histological observation

On the 7^{th}, 13^{th} and 19^{th} day after radiofrequency hyperthermia, 6 rabbits in each group were sacrificed by way of ear venous air embolism. The articular capsule of the knee joint was reopened along the surgery trace, the internal structure of the joint was exposed, and the lateral collateral ligament and the posterior cruciate ligament were cut off to completely separate the femur and the tibia, carefully remove soft tissues around the femoral end, which was detached by a bone knife, with the medial femoral condyle cut off. The condyle was rinsed with running water, placed and fixed in 4% NBF (4 °C) for 24 h. The fixed tissue specimens were rinsed and then placed in 10% EDTA decalcifying solution, which was heated daily in a constant temperature water bath of 60 °C for 10 h. The solution was replaced every other day. It lasted for 6 days until the bone substances became soft to the ideal state (It is appropriate to easily pierce through the bone substances with a pin). And then, the specimens were rinsed with running water, a small piece (0.5 cm × 0.4 cm × 0.3 cm) of tissues (including the cartilage and the subchondral bone) was cut off from the decalcified specimen, dehydrated in an ascending series of ethanol concentrations, cleared in xylene and soaked with paraffin. Afterwards, the cartilage coronal section was placed against the bottom surface of the embedding box for embedding. The tissue specimen wax blocks were discontinuously resected, 6 sections (100 μm space) were taken out from each wax block to be stained with hematoxylin-eosin (HE). Three sections of each tissue specimen were chosen by two pathologists for histomorphological observation. Five different fields of view were selected for each section to mainly observe the pathological changes in knee articular cartilages and subchondral bones of the experimental rabbits at different time points. In addition, score statistics was achieved according to modified Mankins rating rules[7,8] for articular cartilages.

1.5 Real-time quantitative PCR detection of type II collagen

On the 7^{th}, 13^{th} and 19^{th} day after radiofrequency hyperthermia, the total type II collagen in cartilage tissues of each group was extracted and determined by use of quantitative PCR according to the instructions for RT reagent kit (Wuhan ColorfulGene Biological Technology Co., Ltd.) and qPCR reagent kit. RNA in cartilage tissues was extracted through tissue grinding, cell lysis, centrifugation, elution and other steps, and reverse-transcribed by removing genomic DNA and reverse transcription. Applied Biosystems StepOnePlus Real-Time PCR System was applied to the amplification by way of initial denaturation (95 °C, 30 s, 1 cycle), PCR (95 °C, 5 s, 40 cycles), dissolution (95 °C, 5 s, 1 cycle), cooling (50 °C, 30 s, 1 cycle) and other steps.

1.6 Statistical analysis

The measurement data in this study were represented by ($\bar{x}$ ± s). SPSS20.0 statistical software kit was applied to the statistical analysis, with *LSD-Q* test used in this study. The difference $p < .05$ was of statistical significance.

2 Results

2.1 Comparison of histological observation for cartilage tissues among three groups of experimental rabbits

In Radiofrequency Hyperthermia group, the cartilage surface was found to be uneven after 6-day treatment, with the incomplete cartilage fissures observed. The chondrocytes were arranged in a cluster. The matrix staining was normal or mildly faded, and the tidemark was intact (see Figure 3-3). After 12-day treatment, the cartilage surface was found to be damaged slightly, with the chondrocytes clustered. The staining of the matrix was normal or mildly faded, and the tidemark was not intact (see Figure 3-4). After 18-day treatment, the cartilage surface was slightly damaged, the chondrocytes were normally arranged or increased, and the matrix staining was slightly faded. The tidemark was almost intact (see Figure 3-5).

In Lugua Polypeptide group after 6-day treatment, incomplete cartilage fissures were observed, with unclear boundaries. The chondrocytes were arranged in a cluster, the matrix staining was normal, and the tidemark was relatively intact (see Figure 3-6). After 12-day treatment, the cartilage was extensively fibrotic (up to the radiation layer), the chondrocytes were normally arranged or disordered, the matrix staining was slightly faded, and the tidemark was not intact (see Figure 3-7). The cartilage surface was locally fibrotic after 18-day treatment (up to the transitional layer), the chondrocytes were locally numerous and disordered in arrangement, the matrix staining was normal or mildly faded, and the tidemark was not intact (see Figure 3-8).

In Model group, cartilage fibrosis and fissures were observed after 6-day treatment, with unclear boundaries. The chondrocytes were arranged in a cluster, the matrix staining was moderately faded, and the tidemark was intact (see Figure 3-9). Cartilage fibrosis was observed after 12-day treatment (up to the radiation layer), the calcification layer was unobvious, with unclear boundaries. The chondrocytes were reduced, the matrix staining was faded, and the tidemark was not intact (see Figure 3-10). After 18-day treatment, the surface of the cartilage tissues was damaged and fibrotic up to the calcified layer, which was difficult to be identified. The chondrocytes were decreased, the matrix staining was faded severely, and the tidemark disappeared (see Figure 3-11).

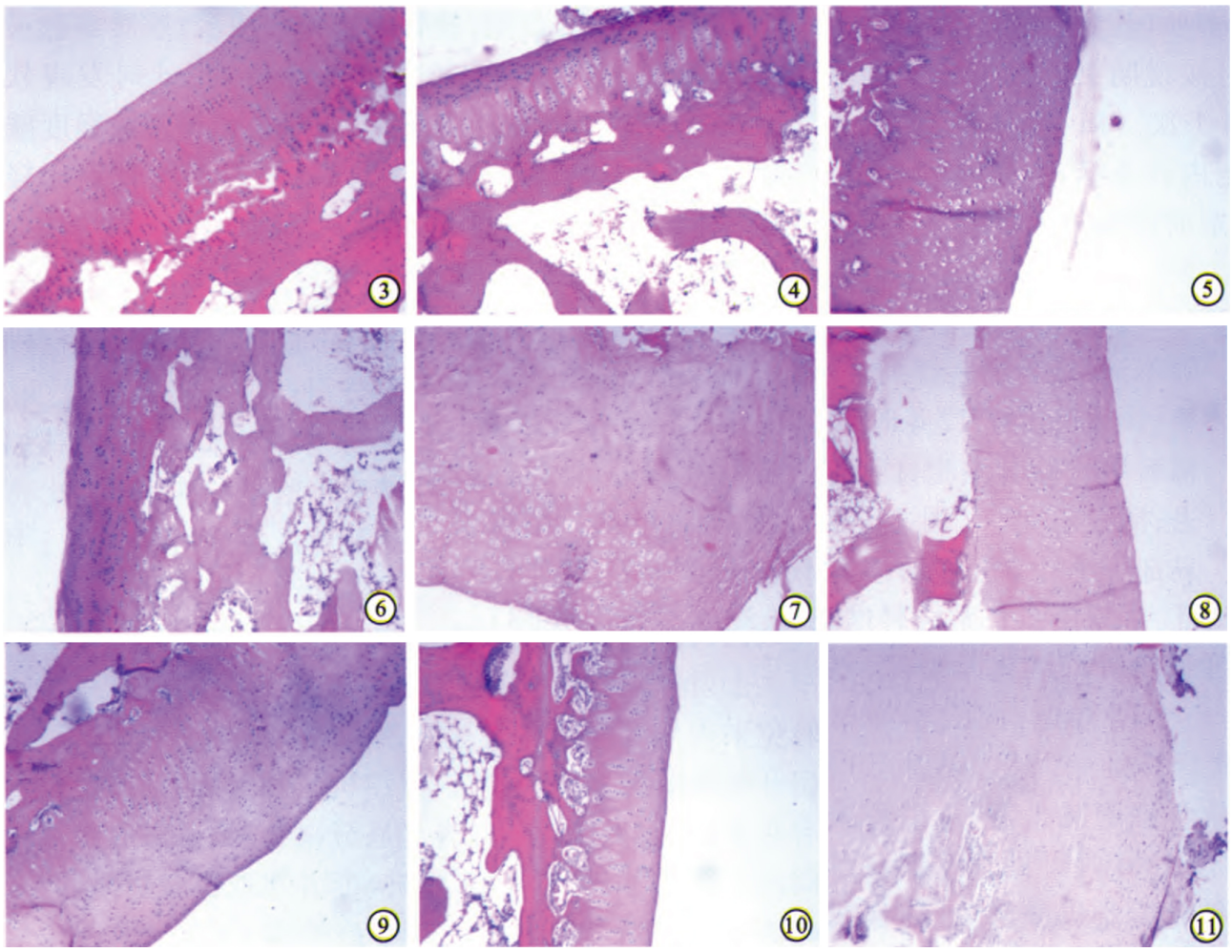

Figure 3: Histological observation of cartilage
Figure 3-3 Histological observation of cartilage in Radiofrequency Hyperthermia group after 6-day treatment (stained with HE, ×100)
Figure 3-4 Histological observation of cartilage in Radiofrequency Hyperthermia group after 12-day treatment (stained with HE, ×100)
Figure 3-5 Histological observation of cartilage in Radiofrequency Hyperthermia group after 18-day treatment (stained with HE, ×100)
Figure 3-6 Histological observation of cartilage in Lugua Polypeptide group after 6-day treatment (stained with HE, ×100)
Figure 3-7 Histological observation of cartilage in Lugua Polypeptide group after 12-day treatment (stained with HE, ×100)
Figure 3-8 Histological observation of cartilage in Lugua Polypeptide group after 18-day treatment (stained with HE, ×100)
Figure 3-9 Histological observation of cartilage in Model group after 6-day treatment (stained with HE, ×100)
Figure 3-10 Histological observation of cartilage in Model group after 12-day treatment (stained with HE, ×100)
Figure 3-11 Histological observation of cartilage in Model group after 18-day treatment (stained with HE, ×100)

2.2 Comparison of Mankins scores in articular cartilage tissues among three groups of experimental rabbits

At each time point after treatment, Mankins scores in articular cartilage tissues were decreased in all the 3 groups, with that of Model group higher than those of the other two groups (Model group > Lugua Polypeptide group > Radiofrequency Hyperthermia group), and the difference was statistically significant ($p < .05$); As to the comparison in the same group, it was found that Mankins scores in Radiofrequency Hyperthermia group were decreased with the prolongation of treatment time, and the difference was statistically significant ($p < .05$). The changes of Mankins scores in Lugua Polypeptide group and Model group showed no

obvious regularities, and the difference in the comparison between different treatment days was not statistically significant ($p > .05$). See Table 1 for specific data.

Table 1: Comparison of Mankins scores in cartilage tissues among three groups of experimental rabbits at different time points after modeling (scores, $\bar{x} \pm s$)

Group	N	After 6 d treatment	After 12 d treatment	After 18 d treatment
Model group	6	$8.50 \pm 1.05^{*\#}$	$9.83 \pm 1.47^{*}$	$11.50 \pm 1.87^{*\#}$
Lugua Polypeptide group	6	6.83 ± 0.75	7.00 ± 0.89	6.00 ± 0.63
Radiofrequency Hyperthermia group	6	$5.17 \pm 0.75^{*}$	$4.50 \pm 1.05^{*}$	$3.50 \pm 0.55^{*}$

Note. In comparison with Lugua Polypeptide group at the same time point, $^{*}p < .05$; In comparison in the same group after 12 d treatment, $^{\#}p < .05$

2.3 Comparison in the expression of type II collagen in cartilage tissues among three groups of experimental rabbits

At each time point after treatment, the expression of type II collagen in cartilage tissues were increased in all the 3 groups, with that of Radiofrequency Hyperthermia group higher than those of the other two groups (Model group < Lugua Polypeptide group < Radiofrequency Hyperthermia group), and the difference between groups was statistically significant ($p < .05$); As to the comparison in the same group, it was found that the expression of type II collagen in Radiofrequency Hyperthermia group was increased with the prolongation of treatment time ($p < .05$); The expression of type II collagen in Model group was decreased with the time going by, but the difference was not statistically significant ($p > .05$); The expression of type II collagen in Lugua Polypeptide group was increased with the prolongation of treatment time, but the difference was of no statistical significance ($p > .05$). See Table 2 for specific data.

Table 2: Comparison of the expression of type II collagen in cartilage tissues among three groups of experimental rabbits at different time points after modeling (scores, $\bar{x} \pm s$)

Group	N	After 6 d treatment	After 12 d treatment	After 18 d treatment
Model group	6	$2.32 \pm 0.25^{*\#}$	$2.05 \pm 0.34^{*}$	$1.65 \pm 0.26^{*\#}$
Lugua Polypeptide group	6	2.48 ± 0.44	2.30 ± 0.32	2.57 ± 0.54
Radiofrequency Hyperthermia group	6	$2.64 \pm 0.60^{*}$	$2.73 \pm 0.19^{*}$	$3.03 \pm 0.45^{*}$

Note. In comparison with Lugua Polypeptide group at the same time point, $^{*}p < .05$; In comparison in the same group after 12 d treatment, $^{\#}p < .05$

3 Discussion

Knee OA is one of the most common joint diseases affecting human health. There is no obvious ethnic or regional difference. The main manifestations are articular cartilage degeneration and secondary hypertrophic osteoarthropathy. As the disease progresses, the patient gradually develops some symptoms such as worsened pain, joint stiffness and limited joint motions, the severe may have difficulty in walking, with the final disability rate of about 53%.[9] Type II collagen is the main component of cartilage matrix, which plays an important role in inducing chondrocyte genesis, differentiation and migration. It is beneficial to the regeneration of articular cartilages.[10] Type II collagen is mainly present in articular cartilages in the body, and its content in other tissues is low. Therefore, the change in cartilage tissues of the body can be estimated by detecting the level of type II collagen.[11] The clinical treatment of knee OA mainly includes drug intervention, non-drug intervention and surgical treatment.[12] The goal of treatment is to relieve pain, improve joint function and life quality, and avoid side effects as much as possible.

Studies have shown that the efficacy of radiofrequency hyperthermia treatment on knee OA is significantly superior to other physical treatment methods.[4] As to in-vitro radiofrequency hyperthermia for knee OA, the mechanism may include: (1) The magnetic field of radiofrequency hyperthermia can inhibit the production of free radicals in the body. During the process of treatment, the heat can increase the local blood flow velocity, enhance the level of metabolism, increase tissue inhibitors of matrix metalloproteinases, and reduce the damage to type II collagen. It is helpful to repair chondrocytes.[13,14] (2) High-frequency electromagnetic oscillation generated by in-vitro radiofrequency can accelerate the exchange of various components in the body, contribute to the improvement of the body's immune function, correct acidosis and promote inflammation resolution, thereby achieving analgesic and antispasmodic effects.[15]

Lugua polypeptide also has a good effect on the treatment of knee OA.[16] Lugua polypeptide mainly contains osteoinductive polypeptide bio-factors, Cucumis melo L. seed ex-

tract, various free amino acids, organic calcium and phosphorus ingredients.[17] It regulates calcium and phosphorus metabolism, increases bone calcium deposition, prevents osteoporosis and has anti-inflammatory and analgesic effects.[18] Related studies have shown that osteoinductive polypeptide biological factors in Lugua polypeptide can effectively promote the synthesis of osteogenic growth factors that affect bone formation and absorption in the body,[19] thus promoting osteoblasts growth, delaying bone degeneration, reducing inflammatory substances and improving local blood circulation. It is one of the commonly used drugs for clinical treatment of bone diseases.

The results of this study showed that at each time point after treatment, Mankins scores in cartilages were decreased in all the 3 groups, with that of Model group higher than those of the other two groups (Model group > Lugua Polypeptide group > Radiofrequency Hyperthermia group), and the difference was statistically significant ($p < .05$), indicating that the experimental rabbits in Model group were the poorest in recovery and Radiofrequency Hyperthermia group was the best in recovery. In addition, at each time point after treatment, it was found that Model group had a lowest content of type II collagen, and Radiofrequency Hyperthermia group had a highest content, further indicating that the therapeutic effect in Radiofrequency Hyperthermia group was the best, followed by Lugua Polypeptide group, and the effect in Model group was the worst. From the above-mentioned results, radiofrequency hyperthermia and Lugua polypeptide have a good effect on the treatment of knee OA, and the therapeutic effect of radiofrequency hyperthermia is more superior. The therapeutic mechanism may be related to the effect of radiofrequency hyperthermia on the improvement of type II collagen content in cartilage tissues.

Conflicts of Interest Disclosure

The authors have no conflicts of interest related to this article.

References

[1] Wu JP, Qiu FZ, Huang JS. Textbook of Surgery. 6^{th} ed. Beijing: People's Medical Publishing House; 2008. 2384 p.

[2] Yang C, Zhou XG. The treatment of knee OA by comprehensive rehabilitation therapy. Chinese Journal of Rehabilitation. 2005; 20(1): 45-45.

[3] Chen CW, Wei XC. The relationship between the changes of articular cartilage collagen and OA. Chinese Journal of Rheumatology. 2003; 7(1): 43-45.

[4] Fu ZH, Jiang RA. Effects of different physical factors on HSS rating of knee OA. Chinese Medical Journal of Metallurgical Industry. 2014; 31(6): 720-721.

[5] Xu Y. Effects of Cervus and Cucumis Polypeptide for Injection on Acute Phase of Knee OA, SOD, NO and MDA. Strait Pharmaceutical Journal. 2013; 25(5): 97-98.

[6] Fang R, Ailijiang A, Lu Y, et al. Construction and pathological features of a rabbit osteoarthritis model in early, middle, and later phases. Chinese Journal of Tissue Engineering Research. 2010; 14(7): 1218-1222.

[7] Mankin HJ, Dofman H, Lipiello L. Biochemical and metabolic abnormalities in articular cartilage from osteoarthritic human hips. J Bone Joint Surg. 1971; 52: 523. https://doi.org/10.2106/00004623-197153030-00009

[8] Wei XC. Articular cartilages. Beijing: Science Press; 2007. 106 p.

[9] Tang X, Zhou Z, Shen B, et al. Serum levels of TNF-α, IL-1β, COMP, and CTX-II in patients with Kashin-Beck disease in Sichuan, China. Rheumatol Int. 2012; 32(11): 3503-3509. PMid: 22068351. https://doi.org/10.1007/s00296-011-2172-8

[10] Chen DC, Zhong CH, Lei J, et al. Study on the treatment of rat osteoarthritis with type II collagen. Biomedical Engineering and Clinical Medicine. 2013; 17(6): 528-533.

[11] Fu ZY, Ouyang HG. Experimental study of the effect of Guyankang granules on the expression of serum type II collagen, TGF-β1, and IL-1β in rats with knee osteoarthritis. Chinese Journal of Osteoporosis. 2013; 19(12): 1275-1277.

[12] Chinese Orthopaedic Association. Guidelines for Osteoarthritis. Chinese Journal for Clinicians. 2008; 36(1): 28-30.

[13] Wang JF, Yang XH, Lin H. Observation and nursing on the treatment of chronic prostatitis by HG-2000 type in-vitro high frequency hyperthermia apparatus. Fujian Medical Journal. 2003; 25(3): 20-20.

[14] Takami M, Lehmann HI, Parker KD, et al. Effect of left atrial ablation process and strategy on microemboli formation during irrigated radiofrequency catheter ablation in an in vivo model. Circ Arrhythm Electrophysiol. 2016; 9(1): e003226.

[15] Fu ZH, Zheng GJ. Observation on the therapeutic effect of in-vitro RF combined with interference current therapy on LDH. Chinese Journal of Physical Medicine and Rehabilitation. 2010; 32(12): 942-943.

[16] Dai ZW. Experimental study on Cervus and Cucumis Polypeptide against articular cartilage degeneration of the knee OA models in rabbit by intraarticular injection. Hubei: Hubei University of Traditional Chinese Medicine. 2011.

[17] Tao HY, Ma YG, Tao HL, et al. Experimental study on the effect of Cervus and Cucumis Polypeptide injection on bone healing. Chinese Journal of Experimental Surgery. 2003; 20(9): 64-65.

[18] Liu GH, Zhang Y, Yang SH. Observation and analysis on the clinical effect of cerLuvus and cucumis polypeptide. Chinese Journal of Hospital Pharmacy. 2004; 24(8): 490-491.

[19] Shang FM, You XJ. Exploration and analysis on clinical application of cervus and cucumis polypeptide. Capital Medicine. 2013; 20(6): 24.